

A close-up photograph of a woven basket filled with fresh, vibrant red raspberries. The raspberries are piled together, with some showing their characteristic bumpy texture and green leafy tops. The basket is made of light-colored, natural fibers. The background is a soft, out-of-focus green, suggesting an outdoor setting. The entire image is framed with rounded corners.

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Full Length Research Paper

Evaluation of the storage stability of pineapple products processed by small and medium scale processing enterprises in Rwanda

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This study aimed at evaluating the effect of storage time on the stability of pineapple products processed by small and medium scale processing enterprises (SMEs) in Rwanda stored at ambient (21 to 25°C) temperatures. Physico-chemical quality characteristics (pH, moisture, fibre, ash, total soluble solids, titratable acidity, sucrose, reducing sugars, total sugars and ethanol) of the sample products were analysed. Changes in the quality were monitored at three and one months interval for syrups, jams and nectars, respectively. Results showed that for nectars, most of the parameters evaluated were stable up to only two months of storage. All nectars, syrups and jams had a pH ranging from 3-5, the normal pH range for fruit products. The levels of titratable acidity of all products were below the maximum limit of 1.35% set by Codex Alimentarius Commission (CAC) Standards. Similarly, their levels of ethanol were less than the maximum of 0.3% recommended by Codex Alimentarius Commission standards. Syrups and jams were stable for most of the parameters up to six months of storage. There was a significant ($p < 0.05$) decrease of sucrose and increase of reducing sugars in syrup and jam samples, specifically from six months of storage. Products from some enterprises exhibited sugar values higher than the maximum recommended by Rwandan, East African and CAC standards. The studied products exhibited significant variations in most of the studied parameters over the storage period with most of the samples not complying with the standards including sugar levels. Processors, therefore, need to be trained on proper fruit processing and the standard requirements for pineapple fruit products.

Key words: Pineapple products, storage stability, chemical properties

INTRODUCTION

Products from tropical fruits have increasingly gained global importance due to their characteristic exotic taste, aroma and colour (Abbo et al., 2006; Bicas et al., 2011). Many products such as juices, jams, jellies, marmalades

and alcoholic beverages are currently produced from various tropical fruits. These include orange, papaya, pineapple, banana, guava and watermelon. Pineapple, being one of the exotic tropical fruits is recognised for its

very pleasant sub-acid, aroma and juicy flesh. Both fresh and processed, pineapple products are source of several nutrients beneficial to human health and are found in retail shops, stores and supermarkets around the world (Chia et al., 2012). In Rwanda, pineapple producers have aggressively targeted the export market of the fresh fruit (Rwanda Horticulture Development Authority, 2008). Small scale processors have benefitted from increased production by adding value to the fruit through processing it into different products including juices, jams, wines and dried slices (Austin et al., 2009). Nevertheless, the processed products tend to be of sub-standard making it difficult for the processors to reach the export market. Consequently, the products are locally commercialised. For these products to reach the export market, they need to comply with national, regional and international fruit product standards (CAC, 1981, 2005a, b, 2009; RS, 2008; EAS, 2000). The quality of a packaged fruit product is a function of the physico-chemical characteristics such as sugars, pH, acidity, fibre, moisture, alcohol, total soluble solids and other chemical constituents as well as organoleptic properties (Ewaidah et al., 1988). In addition, fruit products are highly prone to microbial deterioration if not adequately processed and stored (Osuntogun and Aboaba, 2004). A large number of lactic acid bacteria, coliforms, yeasts and moulds cause spoilage because they are able to ferment carbohydrates and produce undesirable changes such as production of acids, alcohols and diacetyls, which negatively alter chemical and organoleptic properties of the food products (Tribst et al., 2009). Such changes render the products fail to meet standards acceptable to the export market and can cause food related health problems. So far the effect of storage time on the quality characteristics of pineapple nectars, syrups and jams commonly processed by small scale processors in Rwanda has not been studied. The aim of the present study was therefore to evaluate the effect of storage time on physico-chemical characteristics of the pineapple products (nectars, syrups and jams) processed by small and medium enterprises while taking into consideration the requirements of the local, regional and international standards. The findings from this study will be a basis of recommendations on how to produce better shelf-stable marketable pineapple products.

MATERIALS AND METHODS

Sampling

Random sampling technique was used to obtain bottled pineapple nectars, syrups and packaged jam samples at each pineapple

processing enterprise involved in the study. Samples, 62 bottles of 500 ml for syrups, 62 pots of jams and 62 bottles of nectars were collected from each enterprise and coded. Table 1 shows the abbreviations of samples used with their respective codes. For example, s_1 meant syrup number one that is collected from the small enterprise number one and N_4 meant nectar number four that is collected from the medium enterprise number four. The 10 enterprises were scattered across the country and not located at the same place. Jams were collected from only six enterprises, which were processing jams in addition to syrups and nectars. Samples were transported in paper cartons and kept at room temperature ranging from 21^o to 25^oC in the Southern province of Rwanda, Huye district for subsequent analysis.

Physico-chemical characteristics determinations

Chemical and physico-chemical characteristics of the products were determined by Official Methods of Analysis of the Association of Analytical Chemists (AOAC, 1995). Totalsoluble solids were determined by using portable refractometer (ref.8145, 0320v, France); pH by potentiometric method, titratable acidity was determined by dissolving a known weight of sample in distilled water and then titrated against 0.1 N NaOH using phenolphthalein as indicator and expressing the results as percent citric acid. Moisture, dietary fibre and ash contents were determined using the approved Official Methods of Analysis (AOAC, 1995). Total sugars, reducing sugars and sucrose were determined according to Luff-Schoorl method (EAS, 2000) and ethanol by densimetric method (AOAC, 1995).

For analysis, one bottle was randomly selected each month for nectars, and every three months for syrups and jams. All determinations were carried out in triplicates and the mean values were reported. All laboratory analyses were conducted at the Laboratory of Analysis of Foodstuff, Drugs, Water and Toxics (LADAMET) of the Faculty of Medicine at the University of Rwanda (UR) from October 2012 to October 2013. Chemicals and reagents were supplied by Merck company, France.

Statistical analysis

Data obtained from the study were analyzed by Genstat statistical software 14th edition (VSN International Ltd, UK). Analysis of variance (ANOVA) was performed to determine significant differences between the main factors. Means were separated by Tukey's Honest Significant Difference at $p < 0.05$. Data were expressed as Mean \pm SD.

RESULTS AND DISCUSSION

Chemical characteristics of the pineapple products

pH

The results of pH changes in pineapple syrups during 12 months of storage are shown in Figure 1A. There was a

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Table 1. Abbreviations of samples used in this study.

Syrup samples	Enterprise processing syrups	Nectar samples	Enterprise processing nectars	Jam samples	Enterprise processing jams
S ₁	Small	N ₁	Small	J ₁	Small
S ₂	Medium	N ₂	Medium	J ₂	Medium
S ₃	Small	N ₃	Small	J ₃	Small
S ₄	Medium	N ₄	Medium	J ₄	Medium
S ₅	Medium	N ₅	Small	J ₅	Medium
S ₆	Small	N ₆	Small	J ₆	Medium
S ₇	Medium	N ₇	Medium		
S ₈	Small	N ₈	Small		
S ₉	Small	N ₉	Medium		
S ₁₀	Medium	N ₁₀	Medium		

S₁-S₁₀ means Syrup sample; N₁-N₁₀ means Nectar samples; J₁-J₆ means Jam samples.

significant ($p < 0.05$) decrease in pH values for samples coded (S₁, S₂, S₅ and S₆) and significant increase for S₃, S₄ and S₈ samples up to 12 months of storage. There was no observed significant ($p > 0.05$) change in pH values for samples coded S₇, S₉ and S₁₀. The highest and lowest pH values were 3.7 and 2.7.

For jams, it was observed that, the products remained stable as the storage time went up to 12 months of storage. The pH values in all five jam samples did not change significantly ($p > 0.05$) except for J₆ where it decreased significantly ($p < 0.05$) from 3.73 to 3.51 up to 12 months of storage (Figure 1B). pH ranged from 3.0 to 4.4 over storage time. In the case of nectars, there were no significant ($p > 0.05$) changes in pH values for the majority (60%) of nectars except for N₄, N₅ and N₉ where it decreased significantly at two months of storage while it increased significantly for N₃. The pH range was 2.2 to 4.6 (Figure 1C). The highest and lowest pH values of 4.4 and lowest value of 2.2 were respectively observed in nectars as depicted in Figure 1C.

The majority of the samples had a normal pH ranges approaching an estimated pH value of 3.6 of the natural pineapple fruit reported by Nirmara and Reddy (2011) and Camara et al. (1995) and were within the range of 3 to 4 for pineapple products (Tasnim et al., 2010; United States Food and Drug Administration, 2007). Few samples had a pH less than 3.0. This was expected as pineapple fruit pH is known to vary with growing location, harvest time, fruit maturity and other factors, which affect the fruit (Bartolome et al., 1995). The low pH nature of the sampled products was due to the acidic nature of the pineapple fruit used. Fasoyiro et al. (2005) have reported similar pH increase in roselle fruit-flavoured drinks stored at ambient temperature. The authors have suggested that the increase in pH could be due to the decomposition of fermentable substrates especially the carbohydrates in the pineapple fruits and sugars added thereby increasing

the acidity. Similar changes may have also taken place in this study for some products resulting in an increased pH. The increase in pH of some products in this study corroborates with a significant increase of pH in untreated and irradiated pineapple juice stored for 13 weeks reported by Chia et al. (2012).

Insignificant changes in pH values as observed in some products in this study were similarly reported in pasteurised pineapple juice during storage period of 13 weeks (Chia et al., 2012), heated orange juice stored at 22°C for 13 weeks (Yeom et al., 2000) and canned orange juice stored for one year at 24°C (Camara et al., 1995). For some products, there was a pH decrease and similar findings were reported by Jan and Masih (2012) during the storage stability study of pineapple juice blend with carrot and orange juice. pH is one of the important quality parameters that describe the stability of bioactive compounds in fruit juices (Sanchez-Moreno et al., 2006), it is therefore noted in this study that, pineapple syrups, nectars and jams had expected pH values for fruit and juice products during the storage period. Though there was variations in pH levels throughout the storage period of the studied products, the levels of pH of the samples in this study lead to suggest that pineapple fruits used for processing were of acceptable grade.

Titrateable acidity

The results of Total Titrateable Acidity (TTA) for syrups (A), jams (B) and nectars (C) are shown in Figure 2. Total titrateable acidity values did not change significantly ($p > 0.05$) during storage in the majority of the syrups and jams. It changed however significantly ($p < 0.05$) in most of the nectars during storage. Titrateable acidity ranged from 0.32 to 0.8%; 0.3 to 0.7% and 0.0 to 0.9%, respectively for syrups, jams and nectars after 12 months of storage.

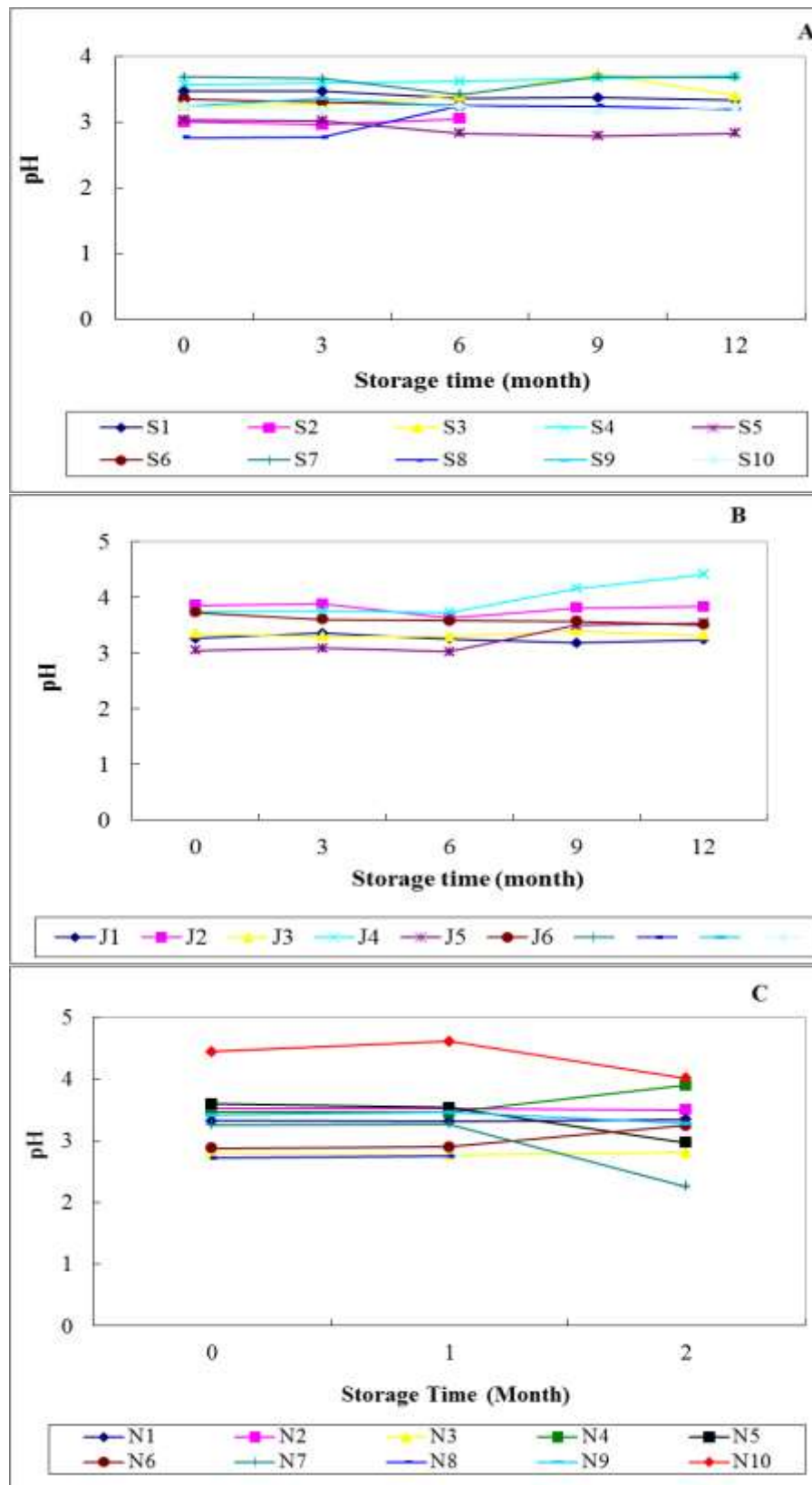


Figure 1. Variation of pH values of (A) pineapple syrups, (B) jams and (C) nectars at different storage times.

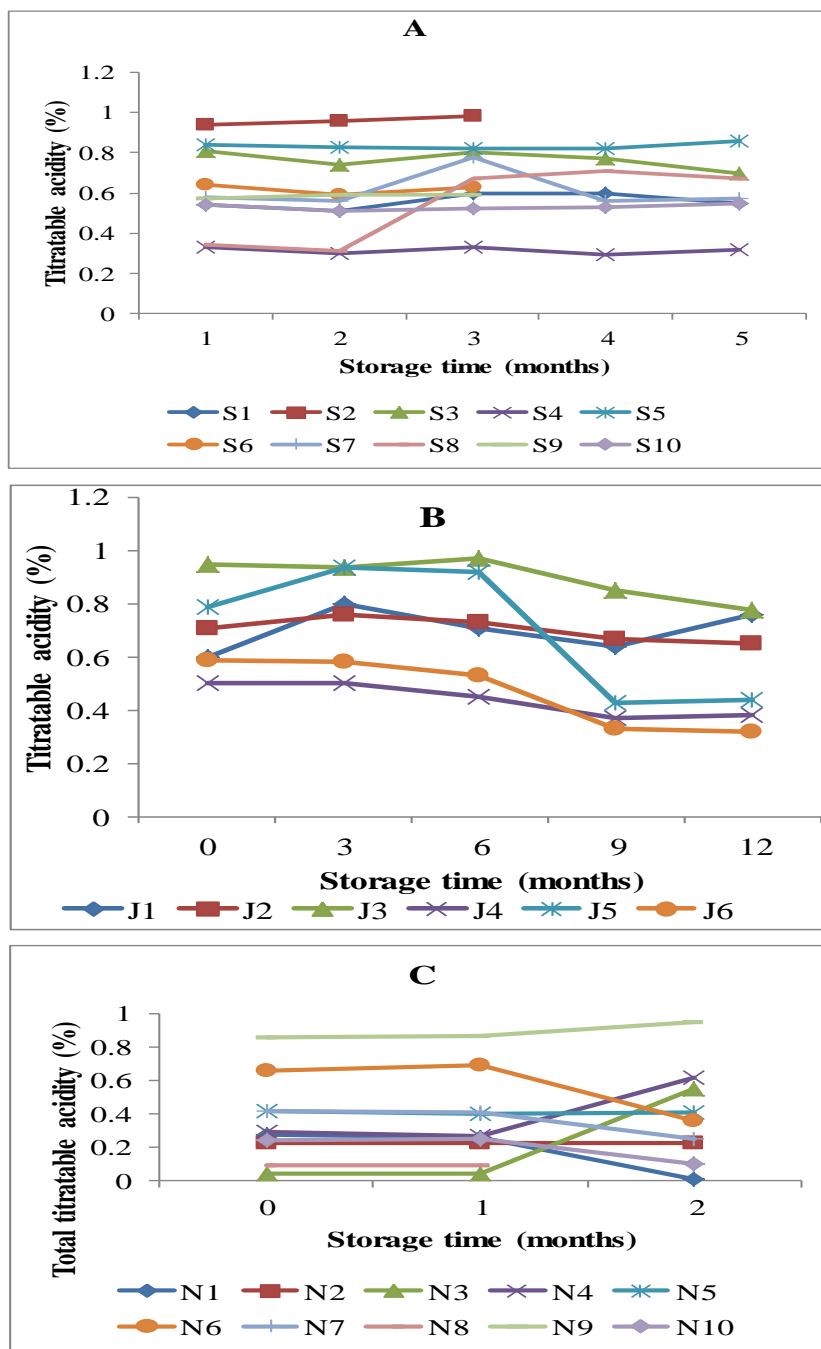


Figure 2. Variation of total titratable acidity values of pineapple Syrups (A), jams (B) and nectars (C) at different storage times.

It increased significantly ($p < 0.05$) up to 12 months of storage for samples coded S₁ and S₈ and it significantly ($p < 0.05$) decreased for samples coded S₃ and S₇ (Figure 2A). Total titratable acidity in jams was stable throughout the storage period but there was a significant ($p < 0.05$) decrease in samples coded J₅ and J₆ (Figure 2B).

Titratable acidity decreased significantly ($p < 0.5$) in nectars coded N₁, N₆, N₇ and N₁₀ while it significantly increased for samples coded N₃, N₄ and N₉. There was no significant ($p > 0.05$) changes in samples coded N₂, N₅ and N₈ as shown in Figure 2C.

Similar stability in titratable acidity was observed in the

thermally pasteurised pineapple juice stored for 13 weeks (Chia et al., 2012) and in grape juice during the storage duration (Buglione and Lozano (2002). The observed significant changes in TTA in some products in the current study were also reported by Ewaidah et al. (1988) in tomato juice stored for one year and Chia et al. (2012) in Ultra Violet (UV) irradiated pineapple juice through the storage period of 13 weeks. Those changes in titratable acidity could be attributed to conversion of acid into sugars (Keditsu et al., 2003). Nevertheless, beside the changes on total titratable acidity, the majority of the products complied with the standards with regards to total titratable acidity whose values were below the maximum limit of 1.35% set by Codex standards for pineapple juice (CAC, 2005b). Therefore, in addition to whatever malpractices that may have been done in the studied samples, TTA values remained close to the values found by Camara et al. (1995) in authentic commercial pineapple juices and nectars, which had legal TTA values.

Moisture

Results for moisture contents in pineapple syrup, jam and nectar are shown in Figure 3 (A, B and C). The results show that, in syrups (Figure 3A), moisture was stable up to six months of storage for samples coded S₁, S₂, S₃, S₅, S₈ and S₉ and the significant changes occurred from the ninth month of storage especially for sample S₆. The rest of the syrups (S₄, S₇, S₁₀) had stable moisture only up to three months of storage. The moisture ranged from 29.5 to 68.0% during 12 months of storage. Moisture content was stable up to three months and started to significantly ($p < 0.05$) either increase or decrease from six months of storage for most of the syrups ranging from 32.63 to 54.66%. Only samples coded S₄ was stable up to 12 months of storage (Figure 3A). In nectars, the moisture ranged from 79.93 to 95.38% and significant changes ($p < 0.05$) started to occur at the second month of storage. All of the nectars had moisture values ranging from 78.45 to 95.36% (Figure 3C).

These values are normal values for fresh fruit and vegetable juices for which a normal moisture values range from 80-95% (Kirk and Sawyer, 1991). The moisture of jams ranged from 31.06% for samples J₆ to 56.1% for J₃ (Figure 3B). It was stable for only sample J₄ throughout the entire storage period of 12 months and for samples J₁, J₂ and J₅ a significant decrease ($p < 0.05$) was observed from the sixth month of storage while for samples J₃ and J₆ a significant increase was observed from the ninth month. These results show inconsistent changes in moisture for different jam sources over the 12 months of storage period. Moisture is one of the indices used to assess the authenticity of the fruit products. The change in moisture content of some samples could be attributed

to either the inappropriate packaging materials (such as packaging not closing tightly), the nature of the packaging material itself in terms of moisture permeability or the change in the structure of the samples (Venir et al., 2007).

Lack of packaging materials has been mentioned as the major constraint in food SMEs business. From these findings, it is observed that significant variations in moisture content started from six months of storage for syrups and jams and from the end of the first month of storage for nectars. Consequently, based on moisture content criterion it is proposed that syrups and jams be stored up to six months and nectars be stored up to one month. Similarly, Alzamora (1993) have found a storage duration of high moisture fruit products ranging from 4 to 8 months.

Fibre

Results showed that samples had very low fibre levels ranging from 0.008 to 0.07%; 0.14 to 0.40% and 0.024 to 0.040% for syrup, jam and nectar samples, respectively at 12 months of storage. The levels of fibre in the studied samples were lower than 5%, which is the minimum limit set by the Codex and East African and Rwandan standards in pineapple fruit juices (EAS, 2000; CAC, 2005a, 2009; RS, 2005). The low levels of fibre content noted in the analysed products was expected because, processed fruit products including juices are known to be low fibre content food products (Kelsay et al., 1979). Dietary fibre comes from the portion of plants that is not digested by enzymes in the intestinal tract (Anderson et al., 2010). Part of it, however, may be metabolized by bacteria in the lower gut. Different types of plants vary in their amount and kind of fibre. Fibre includes pectin, gum, mucilage, cellulose, hemicellulose and lignin (Birch and Parker, 1983). Fruits and vegetables are good source of dietary fibre. Research has shown that a high-fibre diet has several benefits including prevention and treatment of constipation, hemorrhoids and diverticulosis as well as decrease of blood cholesterol (Theuwissen and Mensink, 2008). Pineapple processors are advised to process graded pineapple fruit in order to come up with a product of expected values of fibre content.

Ash

Results showed that ash levels were very low in syrups, jams and nectars. The levels ranged from 0.11 to 1.2%; 0.14 to 0.40% and 0.028 to 0.21%, for syrups, jams and nectars, respectively after 12 months of storage for syrups and jams and after two months of storage for nectars. For syrups, ash levels remained stable up to six months, however, significant ($p < 0.05$) changes were

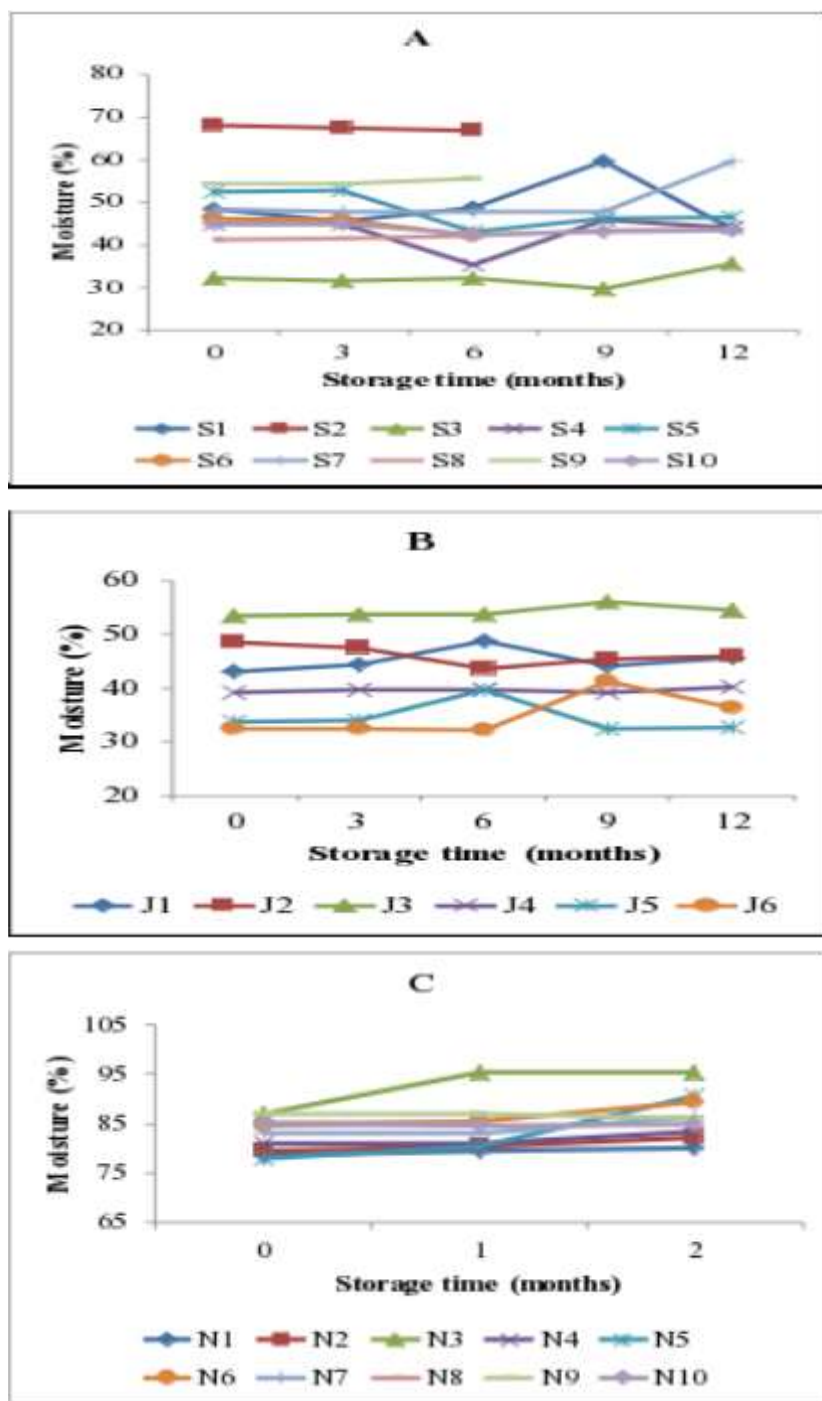


Figure 3. Variation of moisture values of (A) pineapple syrups, (B) jams and (C) nectars at different storage times.

observed from the ninth month of storage. For jams, ash levels were stable throughout the period of 12 months of storage. Only samples J₄ and J₆ were stable up to six months of storage and significant ($p < 0.05$) changes were

observed from the ninth month of storage. For most of the nectars, ash levels remained stable up to two months of storage but for sample coded N₆ and N₉, there were significant decreases at the second month of storage. For

samples N₂ and N₅, the significant decreases were observed at the end of the first month of storage. Ash levels in a food product represent inorganic residue remaining after destruction of organic matter (Ranganna, 1986). The changes observed in ash content for these few samples were expected as it has been reported that some changes in ash content could occur during storage due to some interactions between constituents (Ranganna, 1986). A similar finding of decrease in ash content for stored soursop juice was observed by Abbo et al. (2006). The stability of ash content in most of the studied products was similar to that reported by Akinyele et al. (1990) who found no significant changes in ash content during the processing and storage duration in pineapples and orange juices. The levels of ash in the studied sample were lower than the expected range of 0.3 to 2% reported in literature for fresh fruits and vegetables (Kirk and Sawyer, 1991). However, similar levels of ash were found by Camara et al. (1995) in authentic pineapple juice concentrate packaged in glass bottles and in pineapple nectars. The very low levels of ash in nectars were obvious because they are only made of 40% of natural pineapple juices (Camara et al., 1995). Ranganna (1986) suggested that the low levels of ash in fruit products could be an indication of the absence of adulterants in the sample products. Accordingly, pineapple products in the current study may be considered as authentic with regards to ash content.

Total soluble solids

Results for total soluble solids in pineapple syrups, jams and nectars are presented in Figure 4. For most of the samples, there was no significant ($p > 0.05$) changes in total soluble solids (TSS) levels throughout the storage time except for S₃ where the TSS levels increased significantly from the sixth month of storage (Figure 4A). The levels ranged from 51 to 65° Brix at initial point of storage for syrups. In jams, the TSS levels ranged from 55 to 86° Brix at the initial point except in J₃ with the lowest level of 46.33° Brix and J₆ with the highest level of 86° Brix (Figure 4B). The levels ranged from 14.17 to 20.17° Brix in nectars at the initial point of storage with the exception of sample N₃ which had the lowest level of 6.1° Brix (Figure 4C). However, there was a significant decrease in levels of Brix in N₆ and N₉ at the end of the first month of storage while there was a significant increase of Brix level in N₃ at the end of the first month of storage.

The amount of total soluble solids has been used as an indicator of fruit product quality and authenticity (Camara et al., 1996). The levels of Brix in the nectars were much higher than the levels in the nectars reported by Camara et al. (1996) which ranged from 11.6° Brix to 15.7° Brix. However, Brix levels in nectars were above 12.8 (% v/v),

which is the minimum limit in pineapple nectars set by CAC (2005b). Similarly, the Brix levels of syrups S₄, S₅, S₆ and S₁₀ in the current study were close to the one of authentic commercial juice concentrate in the same study of Camara et al. (1996). Therefore, most of the syrups and nectars in this study may be qualified as authentic with regards to TSS. However, jam samples coded J₄, J₅ and J₆ were the only ones for which TSS was above 60% as recommended by CAC (2009), probably because they were manufactured by successful and experienced medium enterprises, which have been operating more than five years. The remaining jams were qualified as not acceptable as far as TSS was concerned, may be because most of them were small enterprises with little experience in fruit processing. Consequently, processors need to be informed that the quantity of sugars added to pineapple syrups should not exceed 25 g/kg (RS, 2005) and that for jams, there is a need of having 40% of fruits used as ingredient in the final product (CAC, 2009).

Sucrose

Figure 5 shows the variations of sucrose levels in the syrups, jams and nectars during storage. There was a statistically significant ($p < 0.05$) decrease in the sucrose levels during the storage period for jams and syrup samples and a slight decrease started at the end of the first month of storage for nectars. The levels ranged from 9.15% in syrups S₁ to 15.40% in S₄; 6.24% in jam J₃ to 13.48% in J₄ and 0.36% in nectars N₂ to 5.98% in N₂ at the initial storage time. The levels of sucrose in syrups and nectars were much higher than the levels reported by Camara et al. (1995) ranging from 4.13 to 5.51% and 0.21 to 3.58% for syrups and nectars, respectively.

There was a sharp decrease in sucrose levels from the end of the third month for syrups and jams, and the first month of storage for nectars. Ewaidah et al. (1988) reported that the decrease in the sucrose content in canned orange juices stored for one year was due to conversion to reducing sugars. In their study, sucrose was still present for the juices stored at 24°C up to 12 months of storage. Similarly, in the current study, sucrose was still remarkably present in the products stored at a temperature ranging from 21 to 25°C. The storage conditions, such as high temperature have been reported to facilitate the conversion rate of sucrose to reducing sugars and it is suggested that the rate of sucrose hydrolysis is a function of reactants, temperature and acid-catalyst concentration (Babysky et al., 1986).

The current results corroborate with the results of Babysky et al. (1986) who reported hydrolysis of sucrose in apple juice concentrate stored for 111 days. However, the levels of sucrose did not significantly change in the nectars as their shelf life did not go beyond two months. The high levels of sucrose found in this study and

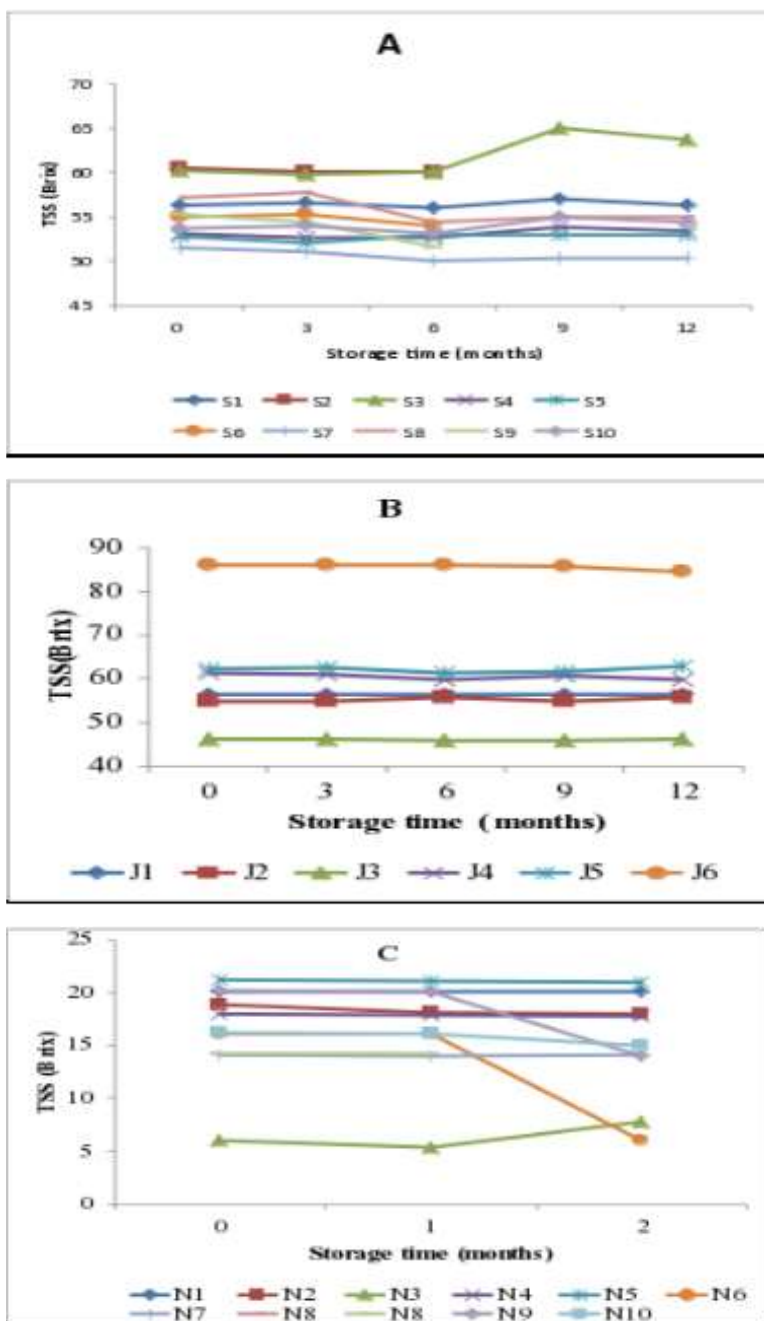


Figure 4. Variation of total soluble solid values of (A) pineapple syrups (B) jams and (C) nectars at different storage times.

variation among product sources could be an indication of an improper addition of sugars during processing.

Reducing sugars

Results of reducing sugars changes over 12 months of

storage for (A) syrups (B) jams and over 2 months of storage for (C) nectars are shown in Figure 6. There was a marked increase in reducing sugars for syrups and jams from the end of the third month of storage. Syrup S₉ had the highest level of reducing sugars (20.10%) and S₁₀ had the lowest levels (15.59%) at 12 months of storage. The observed differences in reducing sugar

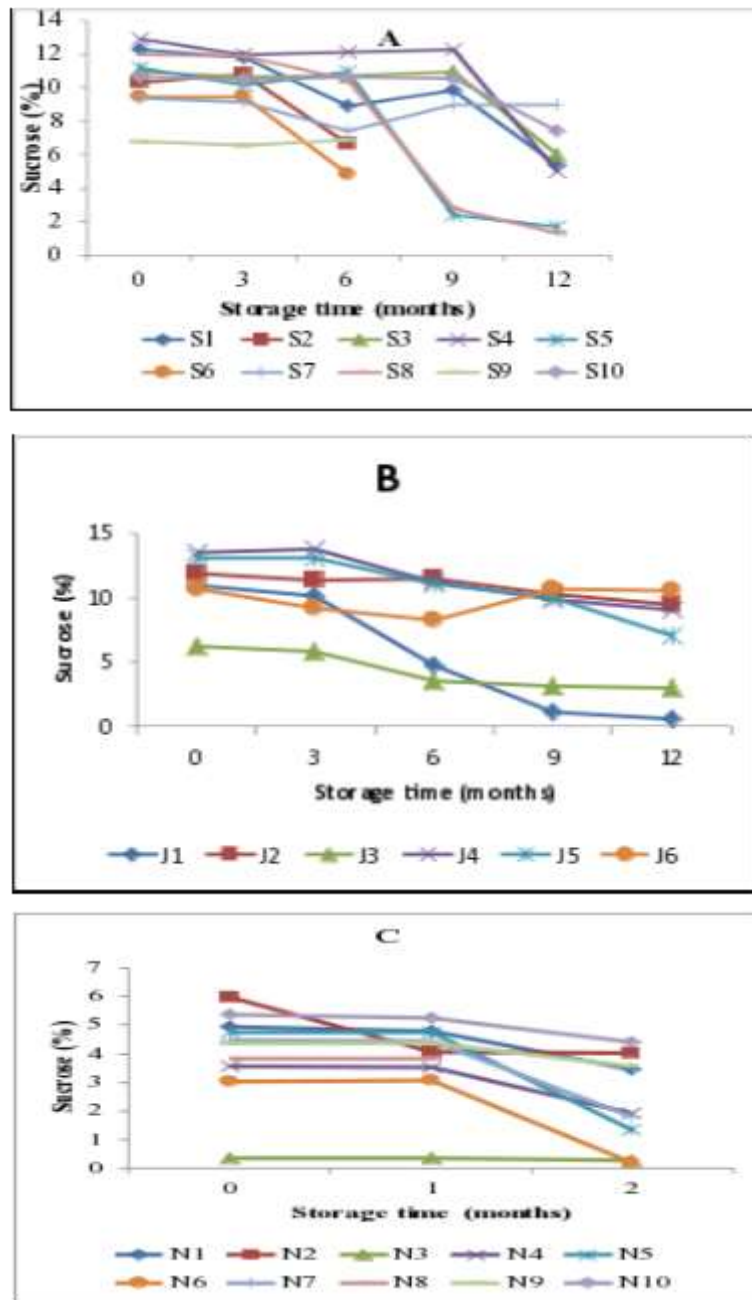


Figure 5. Variation of sucrose values of (A) pineapple syrups, (B) jams and (C) nectars at different storage times.

contents of the samples were expected because samples came from different pineapple growing locations, which is indicated as one of the key factors contributing to the reducing sugar levels in fruit products in addition to the stage of maturity of pineapples to be processed (Tasnim et al., 2010).

For jams, the levels of reducing sugars ranged between 6.6 and 22.00% throughout the storage period. However,

the levels did not significantly change in all the nectars. The rate of increase in reducing sugars ranged between 20 and 70% between the beginning and 12 months of storage. These levels were very low ranging between 0.0031% in N₂ to 0.1% in N₉ at the beginning of storage.

Similar increase in reducing sugars during the storage were also reported in apple juice by Babsky et al. (1986) and in commercial orange canned nectars by Ewaidah

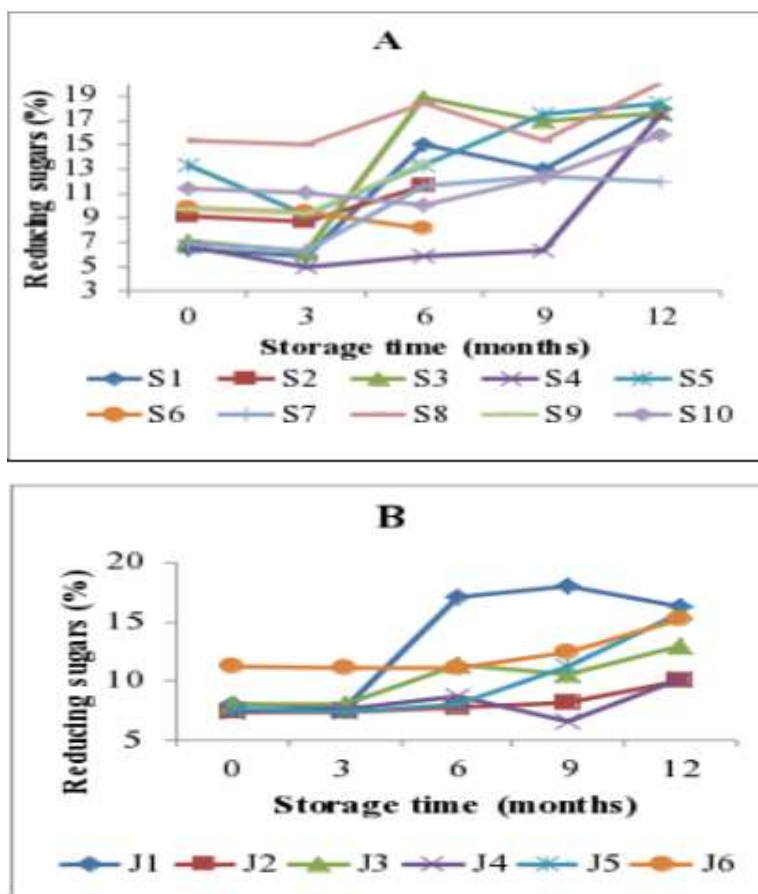


Figure 6. Variation of reducing sugars values of (A) pineapple syrup and (B) jams at different storage times.

(1988). One of the factors that leads to increased reducing sugars during storage of the juices is the decrease in sucrose which hydrolyses into reducing sugars. It was then observed in this study that the increase in reducing sugar levels starting at the end of the third month of storage and onward followed the same trend of decrease in sucrose. Hence, the rate of conversion of sucrose to reducing sugars could have been affected by storage time, temperature and changes in the chemical constituents of the samples.

For the nectars, sucrose and consequently reducing sugar levels did not change significantly over two months of storage. The coefficient of reducing sugars (predominantly glucose and fructose) over sucrose was close to 1 at the beginning of storage for six out of the ten tested syrups. It ranged from 0.89 to 1.43 for samples coded S₁, S₂, S₃, S₄ and S₅. This coefficient of authenticity was also close to one for only two jam samples coded J₂ and J₆. These jams are the only ones that showed insignificant changes ($p > 0.05$) for most of the parameters because they were manufactured by two

successful medium enterprises trained by other different agencies in addition to Rwanda Agriculture Board (RAB) and the National Agricultural Export Development Board (NAEB). The coefficient was much less than one for the remaining five syrups, nectars and most of the jam samples. This could be an indication of the addition of much higher sugars in those samples than the recommended amount. Camara et al. (1996) have suggested that the coefficient of fructose plus glucose /sucrose close to one is a reference index of the authenticity of pineapple fruit products. Therefore, the addition of sugar to some syrups, nectars and jams during processing could have been done inappropriately.

Total sugars

Results in Figure 7 showed that total sugars increased in most of the syrups up to 12 months of storage. The levels ranged from 16.41 to 19.30% in S₇ and 22.95 to 23.70% in S₁₀ at initial point of storage and at 12 months of

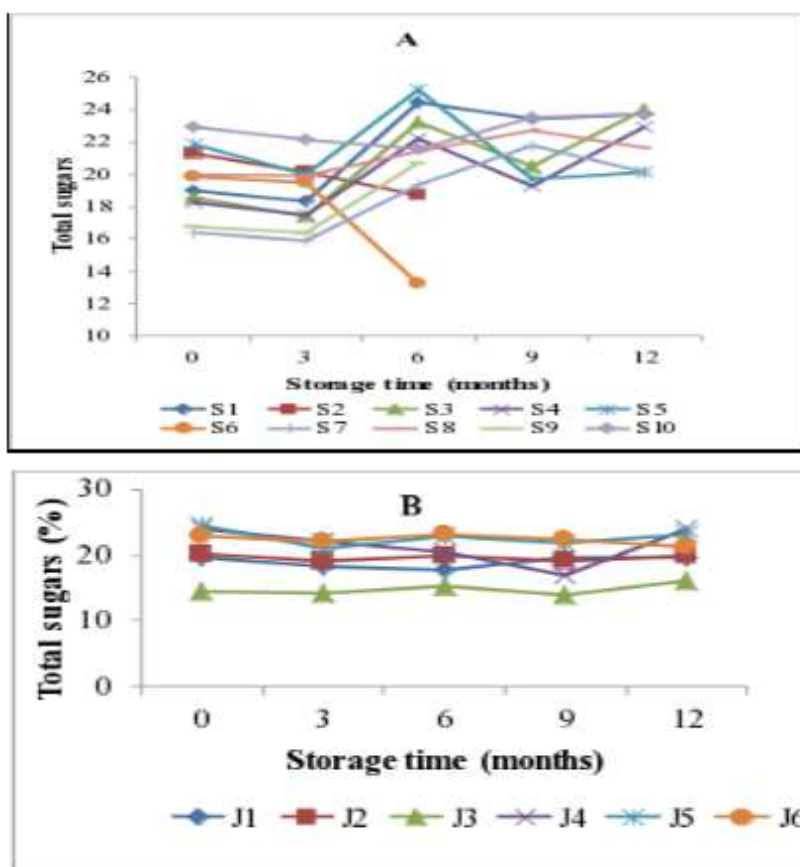


Figure 7. Variation of total sugars values of (A) pineapple syrups and (B) jams at different storage times.

storage, respectively. The increase in total sugars levels started from the third month of storage (Figure 7A). There were also no significant changes ($p > 0.05$) in total sugar content of jams throughout the storage period and the levels ranged from 14.53% in jams J_3 to 24.47% in J_5 at the initial storage time (Figure 7B).

The levels of total sugars in nectars were very low ranging from 0.01% in N_3 to 6.25% in N_9 at the initial stage of storage. They did not change significantly up to the end of the storage period for the majority of nectars.

The increase in total sugars during the storage period in this study followed the same trend of increase in reducing sugars over the storage period reported by Chia et al. (2012). The non-significant changes in total sugars in nectars could also be justified by the non-significant changes observed in reducing sugars over the storage period. The total sugars in this study were higher than the total sugars reported by Camara et al. (1995). Sugar patterns can be used for detecting an inappropriate admixture of sugar solution or fruit juices (Fügel et al., 2005). Consequently, it is possible that more sugar was added in the studied products than the maximum

permissible levels.

Ethanol

The majority of the samples did not have ethanol throughout the storage period. This is a positive aspect for these processed pineapple products as the Codex standards state that ethanol should not exceed 0.3% (CAC, 2005b). Most of the syrups did not have alcohol throughout the storage period. The levels of alcohol above the recommended level were however detected in four out of 10 syrup samples (S_2 , S_3 , S_8 and S_9). Alcohol levels ranged from 0.32 to 1.22% in these syrups. There was no detection of alcohol in all nectars up to the second month of storage. The majority of the jam samples had slightly higher levels of ethanol than the recommended amount at six months of storage. Those levels were in the range of 0.3 to 0.33%. There was no alcohol detected in jams coded J_2 and J_6 from the two successful, well trained and well equipped medium fruit processing enterprises. These observations show that

there was fermentation going on due to low sugar content in some of the products, improper pasteurisation and contamination.

CONCLUSIONS AND RECOMMENDATIONS

The levels of quality parameters for the studied pineapple products vary greatly at all stages of storage indicating absence of non adherence to standards. Nectars were stable for most of the studied parameters throughout the storage time of two months. The fibre content in these nectars did not comply with the standards. Although nectars can be stored up to two months of storage, there is a need of selecting good grade pineapple fruits to use during processing so that the fibre levels are increased up to the required standards of 5%. In addition, proper packaging materials should be used in order to achieve an increased shelf life of the product beyond two months of storage. Jams and syrups were stable up to six months of storage for most of the parameters. Therefore processors need to indicate an expiry date of six instead of the current 12 months. However, their shelf life could also be increased by using good grade pineapple fruits as stipulated in the Codex Alimentarius Commission. Processors should adhere to the requirements stipulated in the standards with regards to the addition of sugar in pineapple products. Future research should investigate the effect of packaging materials on the storage stability of pineapple products.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Bacterial contamination of ready-to-eat meats vended in highway markets in Uganda

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Ready-to-eat (RTE) meats are products sold to consumers which do not require significant further processing except re-heating or completion of cooking process. These meats may constitute a likely potential hazard to human health due to non-compliance with food safety regulations by food handlers. This study was aimed at evaluating the bacteriological safety of RTE roasted meats sold by selected food vendors in Lukaya and Najembe highway markets. Bacteriological analyses were conducted on 20 samples for each of the three meat products which included chicken, beef and goat meat during dry and wet seasons. ISO standard methods were used in the laboratory to test for presence of coliforms, *Escherichia coli* and *Staphylococcus aureus*. Most samples (68.3%) were contaminated and exceeded the recommended microbial contaminant limit (MCL). *S. aureus* was high in beef and chicken where it appeared in 85% of the samples for each product. *S. aureus* was also in 75% of goat meat samples. *E. coli* was high in chicken (50%), followed by beef (45%) and goat meat (35%) samples. Contamination was slightly higher in the wet season. *S. aureus* was the main contaminant. Most RTE meats that are sold in highway markets were highly contaminated. This result should draw the attention of relevant authorities to ensure that adequate hygienic standards and regular monitoring of the quality of RTE meats are improved and practiced to avoid possible foodborne infections.

Key words: Ready-to-eat meats, contamination, coliforms, *Staphylococcus aureus*, highway markets.

INTRODUCTION

Due to the vital role of food in human existence, it is imperative to maintain high level of food safety in order to ensure that human beings are safe from diseases or other related health hazards associated with food (Adolf

and Aziz, 2012). The food vending industry plays a very important role in meeting food requirements of travelers and local dwellers in Uganda as is the case in many developing countries. It feeds thousands of people daily,

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with a large range of foods that are relatively cheap and easily accessible (Tambekar et al., 2008). However, food borne illnesses of microbial origin are a major health risk associated with vended foods (Mensah et al., 2002). The American CDC reported that about 77% of food poisoning occurs in restaurants, 20% in homes and 3% from commercial foods relating to non-compliance with food standards and secondary pollution (Tavakoli, 2008).

RTE foods can be defined as foods and beverages prepared and/ or sold by vendors on the street and in other public places for immediate consumption or consumption at a later time without further processing or preparation (Cho et al., 2011; Tsang, 2002). Due to socio-economic changes characterized by increased mobility, resulting in more RTE foods taken outside the home, food vendors' services are on the increase and responsibility for the food safety have been transferred from individuals/families to the food vendors who rarely enforce good manufacturing practices (Musa and Akande, 2002). Diseases that result from foods are one of the major health problems in developing and developed countries (Razavilar, 2010). Conditions of food safety include efforts to avoid contamination from biological, chemical agents and other substances that can endanger human health (Adolf and Aziz, 2012).

Microbiological food contamination refers to the presence in food of harmful microorganisms which can cause illness (Ahmad et al., 2013). Microbiological safety of food involves assurance that food will not cause microbial harm to the consumer when it is prepared and/or eaten according to its intended use (O'Brien, 2008). There are many risk factors in food vending markets that potentially expose food to microbial contamination. The traditional processing methods that are used in the preparation of vended food, inappropriate holding temperature and poor personal hygiene of food handlers are some of the main causes of contamination of RTE foods (Mensah et al., 2002).

In Uganda, highway vended foods are mostly prepared and sold to travelers at various highway markets by the roadsides. Despite the high potential for outbreaks of food borne illnesses associated with food vending such as cholera, diarrhea, hepatitis and dysentery; improved food safety systems have not been widely implemented in Uganda's food vending markets which raises concern about the role food vending plays in food poisoning (Sebudde et al., 2012). Street foods are frequently linked with gastrointestinal diseases such as diarrhea and typhoid fever due to improper handling and serving practices (Barro et al., 2006; Tambekar et al., 2011). According to Nkere et al. (2011), poor environmental sanitation is largely responsible for much of the contamination and poor personal hygiene among the food handlers. These bacteria can come in contact with the foods when they are prepared especially in unhygienic environments and contaminated cooking utensils

(Shamsuddeen and Ameh, 2008; Kawo and Abdulmumin, 2009).

Gastrointestinal illnesses continue to be a serious public health challenge in Uganda (Muyanja et al., 2011). At least 1.4 million Ugandans are diagnosed with food borne illnesses in Uganda annually (MOH, 2012). This represents 14% of all diseases treated each year (UBOS, 2011). However, this figure is definitely very low for Uganda considering that majority of illnesses in developing countries is treated outside hospitals (at home) and is not recorded at the health centers (MOH, 2013). Therefore, good estimates could put the food borne illnesses beyond 95% of all illnesses in Uganda. The Uganda Demographic Health survey of 2011 reported that 6 in every 10 households experiences a diarrhea episode every month and over 72% of these are attributed to consumption of contaminated food that is accessed from different sources (UBOS, 2011). Markets could be one of the potential sources of microbial contamination. For example in 2013 the Ministry of Health in Uganda reported that 1,357,165 Ugandans were diagnosed with acute diarrhea attributed to consumption of contaminated food. This represented 4.1% of the total population in Uganda and the 5th largest illness diagnosed. In addition, intestinal worms which are also attributed to consumption of contaminated food contribute 5.5% to the disease burden in Uganda. This means that over 2,403,712 Ugandans suffer from intestinal worms annually (MOH, 2013). Cholera and dysentery are also reported by the ministry of health among gastrointestinal disorders that contribute over 730,973 cases every year (MOH, 2012).

According to Karamaji (2012), millions of Ugandans suffer from food poisoning annually. For example in 2012, WHO recorded cholera epidemic in 6 districts across the country. A cumulative figure of 358 cases with 18 deaths were recorded in 5 of the 6 districts. In one district Kasese (in western region) alone, a cumulative figure of 366 cases including 10 deaths were recorded (WHO, 2012). Bwire et al. (2013) noted that Uganda has reported cholera cases to the World Health Organization every year since 1997. They estimated that an average of about 61 to 182 deaths occur in Uganda each year. All these cases have been mainly attributed to eating contaminated food. At the beginning of 2015, a typhoid outbreak was reported in central region of Uganda in which 1940 suspected cases were reported (WHO, 2015). In 2010, there was an outbreak of dysentery in one district (of Kanungu) located in South Western region of Uganda where 12 people were affected (URN, 2010). All these outbreaks are an indicator of the gastrointestinal frequencies and a clear demonstration of the food contamination problem in Uganda.

Several studies showed that different pathogens have been isolated from RTE foods in different countries which include: *Staphylococcus aureus*, *Escherichia coli*,

Salmonella sp., *Shigella* sp., *Klebsiella* sp., *Pseudomonas* sp., *Vibrio* sp., *Campylobacter* sp. and *Listeria monocytogenes* (Adolf and Aziz, 2012; Tavakoli, 2008; Tambekar et al., 2010, Oghene et al., 2014; Makelele et al., 2015; Akusu et al., 2016). The aforementioned observations confirm the risk posed by consuming these vended foods. According to Codex Alimentarius Commission (CAC) classification, cooked RTE foods are among the high risk foods (Tavakoli, 2008). Similarly, Stewart and Humphrey (2002) attributed the cases of food infection and intoxication to poor and inadequate sanitary condition observed in processing of many locally made foods. *E. coli* and *S. aureus* are commonly associated with poor hygiene and sanitation and are usually implicated in the outbreak of food borne illnesses (Odu, 2013). *S. aureus* is capable of producing a highly heat stable protein toxin that causes illness in humans. Onset of symptoms of food poisoning occurs between 1 and 7 days, usually 2 to 4 h after the ingestion of food containing staphylococcal enterotoxins (ICMSF, 2011). The most common symptoms are nausea, vomiting, retching, abdominal cramps and diarrhoea. In severe cases, headache and collapse may occur.

RTE meats are especially a concern since these may be consumed without further cooking and are known to be good growth substrates for pathogenic microorganisms (Zhu et al., 2005). Ensuring good quality raw materials, adequate lethality treatment and effective sanitation of both the equipment and processing environment are crucial in preventing contamination of RTE meats. The presence of pathogens on surfaces of equipment or the environment particularly in post-cooking areas, serves as one of the most important routes for contamination of RTE meats (Zhu et al., 2005). These conditions do exist in Uganda because the state of sanitation and hygiene in Ugandan highway markets is poor. The vendors in these markets lack inadequate cleaning and sanitization materials. They have insufficient knowledge about good hygiene practices. They also lack adequate protective gear such as mouth covers, hair/beard nets and aprons to enhance their personal hygiene. Most highway markets have inadequate hand washing facilities (Winnie, 2005). There are insufficient personnel and therefore a situation of counting money by food handlers during food display and service cannot be avoided. The vendors lack adequate requisite materials and awareness to cover wounds during food handling. The markets do not have adequate latrine/toilet facilities, waste disposal pits/bins and sewage systems. The vending stalls and food storage facilities are also poor (ULRC, 2013). This could expose the meats that are sold in these markets to contamination from *E. coli* and *S. aureus* which are associated with poor sanitation and hygiene.

This study was conducted to evaluate the bacteriological contamination of street-vended RTE roasted meats in highway markets in Uganda. The work

will benefit the unsuspecting consumers, government health agencies and the vendors on minimizing any health risk such food might pose.

MATERIALS AND METHODS

Description of the study area

The study was conducted in Lukaya and Najembe highway markets. Najembe market is located approximately 45 km on Kampala-Jinja highway in the central region district of Buikwe border. Lukaya market is located approximately 100 km on Kampala-Masaka highway in the central region district of Kalungu leading to the southern and western parts of Uganda; and on-ward to the Rwandan and Democratic Republic of Congo (DRC) borders. These markets were also selected for the study because they had a large population of vendors when compared to other highway markets.

Najembe and Lukaya markets have populations of about 320-350 and 350-400 vendors respectively, who are involved in the sale of various foods. Their main activities include mainly roasting and selling of RTE food stuffs to travellers both leaving out of, and coming into Kampala city, Uganda. The food products sold include meats such as chicken, beef and goat meat. Other RTE products sold include cassava, potatoes, plantain "gonjja", chapattis, fruits, vegetables, and drinks such as water, soda and fruit juice.

Selection of products and parameters studied

The study concentrated on high risk RTE products that are sold in highway markets. The high risk foods (chicken, beef and goat meat) were selected basing on the U.S. Food and Drug Administration (FDA) guidelines for identifying high risk foods (FDA, 2013).

Sample collection

Sample collection equipment

A sample collection kit containing an insulated cooler box that was sterilized with ethanol (70%) 10 ice bags and 60 sterile stomacher bags were used to collect and preserve the samples during transportation to the laboratory.

Vendor selection and sample purchase

A total of sixty (60) samples of chicken, beef and goat meat were randomly procured from 30 vendors, in Najembe and Lukaya markets (typically 15 vendors from each market). Twenty (20) samples of each vended meat product (chicken, beef and goat meat) were procured from both markets where 10 samples of chicken, 10 samples of beef and 10 samples of goat meat were obtained each from Najembe and Lukaya markets. Samples were obtained at two separate times, one during the dry season (February 2014) and the other in the wet season (late March 2014); where 5 samples for each food product were obtained in each sampling session from each of the markets. Each market was visited twice and samples of the second visit (wet season) were picked from the same vendors that had provided the samples

Table 1. Compliance of chicken samples to the recommended FAO/WHO and EC microbial contaminant limits (MCLs)

Isolates	Najembe		Lukaya		Total (N=20)
	Dry season (N=5)	Wet season (N=5)	Dry season (N=5)	Wet season (N=5)	
Total coliforms	0(0%)	0(0%)	1(5%)	1(5%)	2(10%)
<i>E. coli</i>	2(10%)	2(10%)	3(15%)	3(15%)	10(50%)
<i>S. aureus</i>	2(10%)	1(5%)	0(0%)	0(0%)	3(15%)

MCL, Total coliforms = < 10 CFU/g; *E. coli* = Absence in 1 g; *S. aureus* = Absence in 1 g. Total samples = 20.

during the first visit (dry season). The actual samples were picked randomly from the roasted meats on display for sell/service to customers since these were regarded as RTE meats/products that required no further processing or preparation. Manual collection was used to pick roasted chicken, beef and goat meats that were found on roasting sticks of selected vendors in the highway markets.

Packaging and delivery

Each category of the procured products was packaged separately. Details of sample history were taken. The sample history captured covered the details on means of delivery of raw food stuffs, time taken after delivery of raw meat before roasting the meat, storage conditions, roasting method, status of water used, time taken by meat out of the recommended temperature range, reheating method and conditions of the surrounding environment. The samples were properly coded, packaged separately in sterile containers and transported in cold pack to the Microbiology Laboratory of the School of Food Technology, Nutrition and Biosciences in Makerere University in Kampala. The transportation time between sample collection and arrival at the Laboratory was approximately 2 hours. Upon arrival, the general physical condition of sampling containers and the samples was noted. The cooler box and the stomacher bags were carefully inspected for tears, pinholes, puncture marks, fractures and loose enclosures before they were accepted. The samples were immediately prepared for analysis.

Sample preparation, culture and bacterial count

The modified method of ISO 4832 and ISO 6888-1 was used for the preparation of the samples. Twenty five grams of each food sample was weighed and homogenized by blending in 225 mL of sterile quarter strength ringer's solution. Thereafter, one milliliter of each food sample homogenate was mixed into 9 mL of the buffered peptone water in a test tube. Serial dilution was made to 10^5 in five other test tubes comprising 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} and 10^{-6} . A 0.1 aliquot portion of each of the diluted samples was spread onto duplicate sterile plates of Baird Parker agar (BPA) and Violet Red Bile Lactose agar (VRBLA) for total aerobic count, *S. aureus* and *E. coli*, respectively. The inoculated plates were incubated aerobically at 37°C for 24 h. After incubation time, the different culture plates were examined for bacterial growth and discrete colonies were counted using the colony counter (Gallenkamp, England) and expressed as colony forming units per gram (CFU /g) of sample homogenate.

To confirm *E. coli*, five (5) colonies of each type were inoculated into tubes of the peptone broth and the tubes were incubated at

37°C for 24 ±2 h. Two to three (2-3) drops of kovac's reagent were added, and formation of a pink ring was a confirmation for *E. coli*.

Statistical analysis

Statistical analysis of data was carried out using one-way analysis of variance (ANOVA) and post-hoc Scheffe tests were used to analyse the level of contamination according to type and source of RTE meats at P≤0.05 level of significance using SPSS version 20 (SPSS Inc., USA). The obtained results for total coliforms, *E. coli* and *S. aureus* were compared with the Microbial Contaminant Limits (MCLs) which are recommended by Codex Alimentarius Commission (CAC) and European Commission (EU). This was intended to determine the samples that were within the recommended limits and those that had exceeded the limits.

RESULTS

Bacterial isolates in chicken, beef and goat meat

Status of contamination of roasted chicken

Overall 10% of all the chicken samples from Lukaya and Najembe that were tested had coliforms which were within the recommended MCL of < 10 CFU/g. None of the samples from Najembe met the recommended MCL while 20% of the samples from Lukaya were below the recommended MCL. Generally, chicken samples obtained from Lukaya market were more compliant than those obtained from Najembe market (Table 1).

Fifty (50%) percent of the chicken samples tested for *E. coli* were within the required MCLs of (absence in 1 g of a sample). Sixty (60%) percent of the samples from Lukaya were within the recommended MCLs, while for Najembe market only 40% of the samples were within the recommended MCL.

For *S. aureus*, only 15% of the chicken samples from Lukaya and Najembe markets were within the recommended MCLs. Only 30% of the samples from Najembe were within the recommended MCLs. No chicken samples taken from Lukaya were within the recommended MCL.

Status of contamination of roasted beef

Of all the samples of beef that were taken from both

Table 2. Compliance of roasted beef samples to the recommended FAO/WHO and EC Microbial Contaminant Limits (MCLs).

Isolates	Najembe		Lukaya		Total (N=20)
	Dry season (N=5)	Wet season (N=5)	Dry season (N=5)	Wet season (N=5)	
Total coliforms	2(10%)	2(10%)	0(0%)	2(10%)	6(30%)
<i>E. coli</i>	4(20%)	3(15%)	1(5%)	3(15%)	11(55%)
<i>S. aureus</i>	1(5%)	1(5%)	1(5%)	0(0%)	3(15%)

MCL, Total coliforms = < 10 CFU/g, *E. coli* = Absence in 1 g, *S. aureus* = Absence in 1 g. Total Samples = 20.

Table 3. Compliance of roasted goat meat samples to the recommended FAO/WHO and EC Microbial Contaminant Limits (MCLs).

Isolates	Najembe		Lukaya		Total (N=20)
	Dry season (N=5)	Wet season (N=5)	Dry season (N=5)	Wet season (N=5)	
Total coliforms	2(10%)	1(5%)	0	1(5%)	4(20%)
<i>E. coli</i>	4(20%)	5(25%)	2(10%)	2(10%)	13(65%)
<i>S. aureus</i>	1(5%)	2(10%)	1(5%)	1(5%)	5(25%)

MCLs, Total coliforms = < 10 CFU/g, *E. coli* = Absence in 1 g, *S. aureus* = Absence in 1 g. Total samples = 20.

markets, only 30% were found to contain coliforms that were within the recommended MCLs of < 10 CFU/g. Samples from Lukaya market were more compliant than those from Lukaya market. Forty (40%) percent of the beef samples taken from Najembe market were below the recommended MCLs compared to 20% from Lukaya market (Table 2).

For *E. coli*, 55% of the beef samples from Lukaya and Najembe markets were within the recommended MCLs. Seventy 70% of the beef samples from Najembe were within the recommended MCLs whereas 40% of the samples from Lukaya were within the recommended MCL.

As for *S. aureus*; only 15% of the beef samples from Lukaya and Najembe markets were within the recommended MCLs and 20% of the beef samples from Najembe were within the recommended MCLs. Only 10% of the beef samples from Lukaya were within the recommended MCLs.

Status of contamination of roasted goat meat

Overall, 20% of the goat meat samples from Lukaya and Najembe markets were within recommended MCLs of <10 cfu/g of a sample for coliforms. Thirty (30%) percent of the goat meat samples from Najembe were within the recommended MCLs while only 10% of the goat meat samples from Lukaya market were within the recommended MCLs (Table 3).

For *E. coli*, 65% of goat meat samples from Lukaya and Najembe markets were within the recommended MCLs. Ninety (90%) percent of the goat meat samples from Najembe market were within the recommended MCLs

whereas only 40% of goat meat samples from Lukaya were within the recommended MCLs.

Concerning *S. aureus*, only 25% of the goat meat samples from Lukaya and Najembe markets were within the recommended MCLs. Thirty (30%) percent of goat meat samples from Najembe market were within the recommended MCLs and 20% of the samples from Lukaya were within the recommended MCLs. In general, Najembe market had more goat meat samples that complied with the recommended MCLs than Lukaya market.

Difference in Coliforms and *S. aureus* contamination of meat products

***S. aureus* contamination between chicken, beef and goat meat**

Goat meat samples had the lowest mean counts for *S. aureus* (3.44 Log₁₀ cfu/g), these were followed by counts of chicken samples (3.99 Log₁₀ cfu/g), and beef meat samples (4.37 Log₁₀ cfu/g) in that order (Figure 1). The difference within the mean counts of the meat products was not significant (P>0.05).

Coliform contamination between chicken, beef and goat meat

Beef samples had the lowest mean counts for coliforms (3.86 Log₁₀ cfu/g), these were followed by goat meat samples (3.94 Log₁₀ cfu/g), and chicken samples (4.37

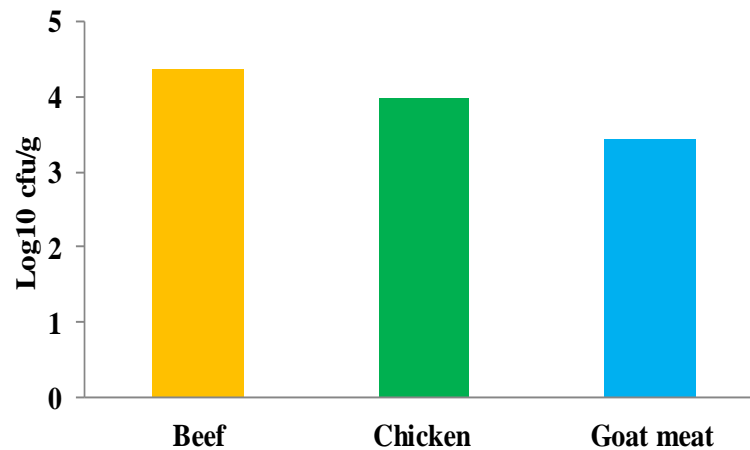


Figure 1. *S. aureus* counts for chicken, beef and goat meat samples.

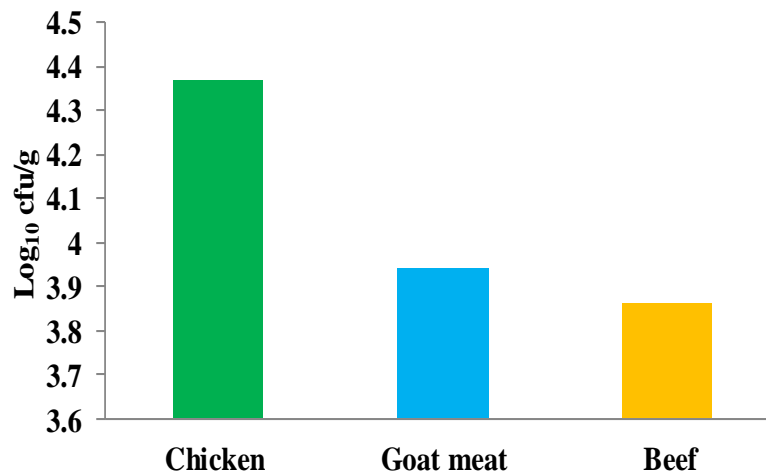


Figure 2. Total coliform counts for chicken, beef and goat meat samples.

Log₁₀ cfu/g) in that order (Figure 2). The difference within the mean counts of the meat products was not significant ($P>0.05$).

Comparison between samples obtained in the dry season and wet season

Total coliforms

During the dry season, chicken samples had lower mean counts (4.05 log₁₀ cfu/g) than wet season (4.68 log₁₀ cfu/g). As for beef samples, dry season had higher mean counts (3.96 log₁₀ cfu/g) than wet season (3.766 log₁₀ cfu/g). For goat meat, dry season had lower mean counts (3.88 log₁₀ cfu/g) than the second round of sampling -wet season (4.02 log₁₀ cfu/g) (Figure 3). Generally, the dry

season had lower counts than the wet season especially for chicken and goat meat although the difference was not significant between the dry and wet seasons ($P>0.05$).

S. aureus

During the dry season, chicken samples had lower mean counts for *S. aureus* (3.15 log₁₀ cfu/g) than the wet season (4.82 log₁₀ cfu/g). For beef samples, the samples of-dry season had lower mean counts (4.59 log₁₀ cfu/g) than the wet season (4.78 log₁₀ cfu/g). As regards goat meat samples, the samples of dry season had higher mean counts (3.69 log₁₀ cfu/g) than wet season (3.18 log₁₀ cfu/g) (Figure 4). Generally, the dry season had lower counts than the wet season although the mean

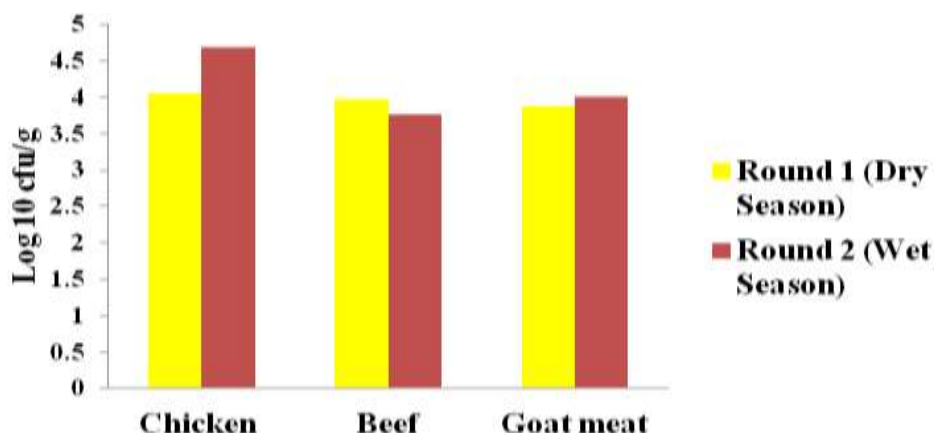


Figure 3. Comparison of coliform counts between chicken, beef and goat meat samples obtained in the first round (dry season) and second round (wet season) of sampling.

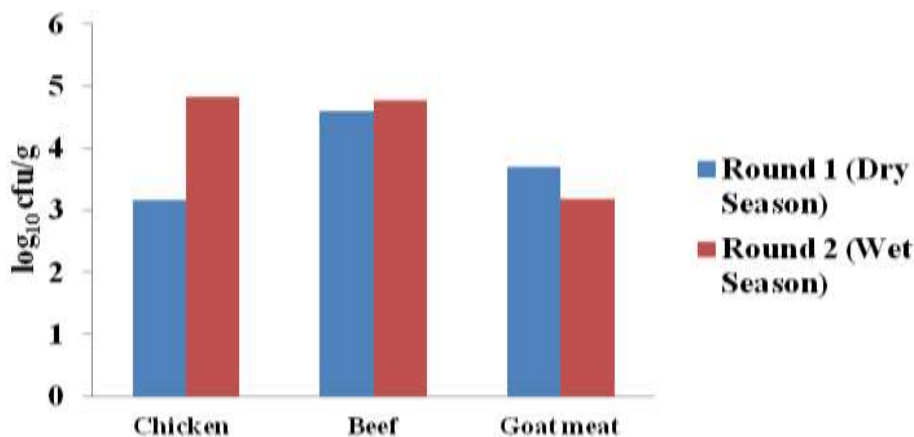


Figure 4. Comparison of *S. aureus* counts between chicken, beef and goat meat samples obtained in the first round (dry season) and second round (wet season) of sampling.

counts of *S.aureus* were not significantly different ($P>0.05$).

DISCUSSION

Bacterial contamination of roasted chicken, beef and goat meat samples as compared to the recommended CODEX and EC Microbial Contaminant Limits (MCLs)

Roasted chicken samples

The occurrence of *E. coli* and *S. aureus* in all the samples may be as a result of poor handling and storage methods used by the food vendors. Similar results have been obtained in previous studies (Wogu et al., 2011; Bukar et al., 2010). The high coliform count in roasted

chicken samples could be attributed to use of dirty items by the vendors to serve or to store the RTE products, overcrowding of markets, long stay of RTE products in the temperature danger zone (5 to 57°C) and the poor hygiene conditions of markets. A comparable study on the risk factors for contamination of RTE street vended poultry dishes in Dakar, Senegal found out that most of the vendors used dirty buckets, sinks, dishes and tongs to serve food to their customers. This exposed the consumers to a risk of eating poultry products that are contaminated with coliforms (Cardinale et al., 2005).

The presence of *E. coli* in chicken samples is an indication of faecal contamination probably at one stage of preparation or from the materials used (Adu-Gyamfi et al., 2012). Handling food with unwashed hands may result in cross-contamination, hence leading to introduction of microbes on safe food (CAC, 1997).

Defective hand washing can facilitate the transmission of pathogenic bacteria found in the environment and on people's hands via food to humans (Chirag, 2013). These scenarios could have been the cause of *E. coli* contamination in the chicken products obtained from the markets.

S. aureus contamination in RTE poultry normally results from excessive handling, man's respiratory passages, skin and superficial wounds which are common sources of *S. aureus* (Pointon et al., 2008). *S. aureus* is a normal flora of the human skin, nasal passage and throat of most healthy people and may have entered the food chain through such sources which suggests poor hygiene practices of the operators. When *S. aureus* is permitted to grow in foods, it can produce a toxin that causes illness. Although it may be destroyed by heat, its toxin is heat stable (Hazariwala, 2002). The presence of these organisms could cause mild to severe symptoms of diseases such as diarrhea, typhoid and cholera (Miriam et al., 2012; Mbah et al., 2012). Contamination of RTE chicken with *S. aureus* could lead to food poisoning and this could be attributed first to non-adherence to standard hygiene practices employed during food preparation and second, the type of water used in mixing the food which is often not clean (Ahmad et al., 2013). Open-air markets have been implicated in direct transfer of *S. aureus* during handling between traders and consumers of RTE foods (Amusan et al., 2010). These factors could as well have been responsible for the *S. aureus* contamination in the chicken samples obtained from the markets.

Roasted beef

Keeping of beef at ambient temperature aids the growth of coliforms to unacceptable levels hence causing the meat's quality to deteriorate (Soyiri et al., 2008). This scenario applies to the current study as well. Same explanation was given for microbial contamination of beef in Kigali, Rwanda where most beef vendors stored beef at ambient temperature for the next day's sale. Most of them did not wash their food stuffs (Eugène et al., 2013). Given the presence of such poor practices among beef vendors in the markets involved in the current study; this could explain the low level of compliance of the beef samples that were tested for coliforms.

A study on street vended foods in Atbara City in the Naher Eneen state of Sudan showed that the most prevalent bacteria contaminating RTE food of beef category was *E. coli* (Abdalla et al., 2009). The isolation of this pathogen in raw and RTE foods such as beef has also been reported by Soyiri et al. (2008), in food vending markets in Accra, Ghana where it was pointed out that it is highly likely that beef vending markets will usually be implicated in shortage of hand washing facilities. From

these comparable studies it is noted that having functional hand washing facilities is critical to the control of *E. coli*. Given the limited availability of such facilities in the highway markets sampled during the study; these factors could as well be responsible for the presence of *E. coli* in the beef samples.

The presence of *S. aureus* in beef samples could have been due to vendors spending long hours with their beef products before they are sold. This increased the risk of excessive human handling and consequent multiplication of *S. aureus* before sale (Eugène et al., 2013). Another related study conducted by Adu-Gyamfi and Nketsia-Tabiri (2007), in Ghana showed that lack of good personal hygiene practices was the major factor contributing to the contamination of beef meat with *S. aureus*. *S. aureus* can be introduced when beef products are exposed to excessive human handling (Adzitey et al., 2011). These factors could also be responsible for the contamination of beef products with *S. aureus* in the highway markets in Uganda given that poor hygiene practices were observable in these markets during the study.

Roasted goat meat

Improper sanitation and hygienic practices such as poor storage of meat products and use of unclean equipment's/utensils during vending can considerably increase the contamination of goat meat with coliforms (Hirwa, 2010). Such practices could have been responsible for the contamination of goat meat products with coliforms.

Mensah et al. (2001) found out that *E. coli* in goat meat was largely due to infestation of flies. Another study by Haque et al. (2008) observed that high levels of contamination from *E. coli* was due to the growth of the existing microorganisms encouraged by the warm temperatures and the cross-contamination from utensils during goat meat preparation. According to CAC (1997), cross-contamination from pests such as flies is a high risk factor to meat handlers because they can facilitate *E. coli* to go directly into the food thus reducing its microbial quality. This could have been the case for goat meat vendors in the markets studied given that goat meat vendors had similar sanitation and hygiene challenges.

A study of bacterial contamination in goat meat from metropolitan Accra, Ghana found out that *S. aureus* was among the most prevalent bacteria contaminating goat meat products (Mensah et al., 2001). The presence of this pathogen in goat meat was found to be due to congestion of food vending stalls and poor personal hygiene practices among the vendors such as irregular hand washing, and absence of proper dressing gear such as gloves, mouth and hair covers and aprons. From this study, it is noted that ensuring personal hygiene and

decongesting meat preparation areas are critical to the control of *S. aureus* in meat vending markets. Given that goat meat vendors in the highway markets studied had congested stalls and did not observe proper personal hygiene practices, the contamination of their goat meat products with *S. aureus* could be attributed to this as well.

Difference in coliforms and *S. aureus* contamination of meat products

The difference between the mean counts of chicken, beef and goat meat samples that were obtained from highway markets was not significant. Related studies testing *S. aureus* in chicken, beef and goat meat at different vending outlets of Lahore, Pakistan also showed that the difference between the mean counts of the three products was not significant ($P > 0.05$) (Ahmad et al., 2013). That study indicated that the slight difference was due to excessive human handling of beef products and limited knowledge about personal hygiene practices among the beef meat sellers as compared to the traders of other types of meat products (goat meat, chicken, mutton and turkey). The current study also found out that beef was supplied at ambient temperature long before the start of business which could have favoured *S. aureus* to grow. Given that these poor hygiene practices were also observed among beef vendors in the areas sampled (highway markets); these factors could also be responsible for the slight differences in *S. aureus* contamination between chicken, beef and goat meat samples.

The coliform counts were higher in chicken samples than in beef and goat meat. A related study on prevalence of different microbial contaminants in meat products from the greater Washington, D.C area also found high coliform contamination of chicken compared to the other meat products (Cuiwei et al., 2001). The study indicated that chicken products were more vulnerable to contamination because chicken contains more fluid than beef and goat meat which could facilitate the quick multiplication of coliforms. This factor could have been responsible for the slightly higher coliform counts found in chicken samples that were obtained from the highway market that were studied.

Comparison between samples obtained in the dry and wet season

The coliform counts during the wet season especially for chicken and goat meats were higher than those of the dry season. During the wet season, the conditions of the surrounding environment and the observed appearance (especially colour) of water used for food preparation had changed when compared with what was observed in dry season. Insect vectors were observed at most stalls. The

environment was filled with mud, stagnant water and the smell in some areas of the markets was more intense in the wet season than in dry season. In addition, most food contact surfaces were observed to be wet. This could explain why the coliform counts during the wet season, especially for chicken and goat meats, were higher than those of the dry season. It is already known that in such scenarios the pathways for contamination include distinguishable vectors such as insects and environmental conditions such as rains and winds (Barro et al., 2007). Use of contaminated water could aid contaminants to enter into the food, hence putting the health of consumers at risk (Chirag, 2013). Unhygienic surroundings and inadequate supply of clean water attract all kinds of flies which further increases food contamination (Chumber et al., 2007).

Results from the samples of chicken, beef and goat meat showed an increment in the counts during the wet season. A comparable study on the bacteriological status of street vended foods of Buldana District, MS, India where samples were picked during the initial rainy season indicates that almost 70% of the food samples collected from street vendors had high bacterial load compared to samples which had been picked during the dry season (Garode, 2012). According to FDA (2016), although food handlers are the main source of contamination from microbial food poisoning outbreaks; wet, muddy and dirty environmental surfaces can also be sources of contamination with various microbes. This means that the rainy season which is characterised by environmental conditions described by FDA could have had an incremental impact on the *S. aureus* counts of the products obtained from highway markets.

Conclusion

The study results indicated that the RTE meats were contaminated with bacterial pathogens such as *S. aureus* and *E. coli* and did not meet the required safety levels recommended by Codex Alimentarius and European Commission (EC). The presence of these food pathogens in the foods could pose a serious public health hazard to unsuspecting consumers as all these bacterial pathogens have been implicated in food borne illnesses. The detection of these organisms in all the RTE meats investigated portends danger that could be associated with poor personal hygiene, poor food preparation, lack of good manufacturing practices, as well as and non-compliance to Hazard Analysis and Critical Control Points (HACCP) principles during the preparation, packaging and serving of these foods to consumers. *S. aureus* contamination was higher in beef meat samples, followed by chicken and goat meat samples, respectively. *E. coli* contamination was higher in chicken samples, followed by beef and goat meat samples respectively although in both cases the differences were not significant. As

expected, samples taken during the wet season were more contaminated than those that were picked during the dry season; although the difference was insignificant. Generally the study findings indicate that the meats that are vended in highway markets are not safe and could pose health risks to consumers. This therefore calls for installation of proper facilities, awareness of vendors in good sanitation and hygiene practices and enforcement of sanitation and hygiene. More so, the following primary food safety measures should be effectively observed by food handlers and vendors: Proper hand washing practices, preparation and selling of foods in hygienic premises, proper covering of prepared foods, washing of utensils and dish with soap, use of portable water as well as proper disposal of wastes among others.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Nutritional value of a dietary supplement of *Moringa oleifera* and *Pleurotus ostreatus*

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Moringa oleifera and *Pleurotus ostreatus* are widely used as food or food supplements. They are demonstrated to have many beneficial effects on nutritional status and human health. The aim of the present study was to evaluate the nutritional value of *M. oleifera* and *P. ostreatus* mixture in specific proportions. The mushroom species was cultivated at the Mushroom Biotechnology Laboratory and *M. oleifera* at the botanical garden of the University Cheikh Anta Diop de Dakar, Senegal. The compost of *P. ostreatus* culture was corn and straw peanut. Mixtures of different proportion powders of *M. oleifera* and *P. ostreatus* were used for investigations. Results showed that the product contain 35.08% crude proteins, 14.28% carbohydrates, 22.71% fat, 20.96% fibers and 6.98% of total ash. The energy was 401.79 kcal for 100 g of dry matter. Among minerals, potassium (1566.83 mg/100 g) are the most abundant mineral element followed by phosphorus (318.55 mg/100 g), calcium (284.33 mg/100 g) and magnesium (253.14 mg/100 g); the less abundant was copper with 0.53 mg/100 g. This study shows that the used proportion of *M. oleifera* leaves and *P. ostreatus* powders mixture could be a good source of minerals, proteins and carbohydrates.

Key words: *Moringa oleifera*, *Pleurotus ostreatus*, dietary supplement.

INTRODUCTION

Many people living in developing countries all over the world suffer of hunger -12.9% of the population is underfed. Sub-Saharan Africa is the region with the

highest prevalence of hunger. About 25% of the populations suffering from chronic malnutrition live in sub-Saharan Africa (FAO, 2015). Every one child on six in

developing countries, so like 100 millions of children, is suffering of insufficient weight (WHO, 2010). In the world, 66 millions of children go to school with an empty belly and of which 23 million live in Africa (WFP, 2012). According to World Food Program (WFP, 2012), reported that annually 3.2 billions of dollars for feeding 66 millions of children who go to school. However, according to current's provisions, by 2020, Africa will have the most increasing percentage of death because of non-communicable diseases. Among the risk's factors, bad feeding and a poor consumption of fruits and vegetables could be the cause of 1.7 million of death (WHO, 2010). In order to fight hunger and diseases, many organisms and governments, encourage the use of vegetal species as minerals and proteins sources (FAO, 2015). In some developing countries like Senegal and Cameroon, governments encourage the use of naturals and bio-products against under-feeding which cause many infantile mortality and morbidity (UNICEF, 2007; Shetty, 2010). Many studies have been shown the health and nutritional interest of edible mushrooms (Zhang *et al.*, 2016; Alam *et al.*, 2008; Pornariya and Kanok, 2009). Mushrooms and some plants provide proteins, carbohydrates, minerals, fibers, vitamins, minerals and fatty acids (Khatun *et al.*, 2012; Okwulehie *et al.*, 2014; Barros *et al.*, 2007). They have therapeutic properties and many of them have been used in medicine all over the world (Badalyan, 2014). Researchers have shown that *P. ostreatus* has antitumor effects, antioxidant properties, antihyperlipidemic effects, antidiabetic effects, (Zhang *et al.*, 2016; Abrams *et al.*, 2011; Alam *et al.*, 2008; Elmastas *et al.*, 2007; Jayakumar *et al.*, 2007; Jayakumar *et al.*, 2006). Also, the plant *M. oleifera* has multiple therapeutic effects (Farooq *et al.*, 2007; Ferreira *et al.*, 2008; Sholapur and Patil, 2013). High malnutrition rates are common in the pastoral regions in different parts of the world. In order to solve this complex problem, simple alternative solutions that contribute considerably to immediate food self-sufficiency are required. The objective of the present study was to determine the nutritional value of *Moringa oleifera* and *Pleurotus ostreatus* mixture in specific proportions.

MATERIALS AND METHODS

Plant material

The mushroom *P. ostreatus* was cultivated and harvested at the Biotechnology Laboratory of mushrooms at the University Cheikh Anta Diop de Dakar (UCAD), Senegal. They have obtained on a substrate from straw peanut and rice bran; the mycelium was

inoculated on corn seeds. Fresh leaves of *M. oleifera* were collected from the Botanical garden of UCAD, identified and authenticated by a taxonomist from the Laboratory of Botany and Plants systematic, UCAD.

M. oleifera and *P. ostreatus* powder mixture preparation

Carpophores of *P. ostreatus* were washed with distilled water and then heated at 50°C for 10 min (Manzi *et al.*, 2004). They were drained and dried at 40°C till a dry texture was obtained which was then molded in a sterilized at 170°C for 30 min local molding machine in order to obtain a fine powder. The leaves of *M. oleifera* were washed thoroughly two times with distilled water, and put inside the bag containing 1% of sodium hypochlorite for 5 min as describe by Bénissan *et al.* (2012). They were washed again with distilled water and dry on tissue paper in a clean room sheltered from the sun during 7 days. The dry leaves were then reduced to get powder for further exploration by an artisanal machine after sterilization at 170°C during 30 min (Rutala *et al.*, 2008). According to the recommendation of FAO (2015), WHO (2007) and Goyens (2009), one mixture of two powders (*M. oleifera* and *P. ostreatus*) was prepared in function of the recommended daily intake under the aseptic conditions. These studies notified that when people take 30 g of *M. oleifera* and 15 g of *P. ostreatus*, more than 50% of daily recommended intakes of total proteins, calcium, potassium, magnesium, phosphore, iron, manganese, copper, zinc are covered by these proportions. For that reason, a mixture with these two species according to these recommendations was made.

All metallic serving utensils were sterilized with local autoclave at 170°C for 30 min, plates in autoclave at 121°C for 20 min, the others that didn't support high temperature were sterilized at UV for 30 min.

Method of analysis

Chemical composition analysis

Chemical composition of sample (*M. oleifera* and *P. ostreatus* mixture powder in 2:1 proportions) was determined for moisture, crude protein, total fat, minerals (sodium, calcium, magnesium, phosphorus, potassium, iron, copper, zinc, manganese). Chemical analysis of samples was conducted at the "Laboratoire National d'Analyses et de Contrôle" in Dakar (Senegal), at Institute of Medical Research and Medicinal Plants studies MINRESI and "Centre Pasteur du Cameroun". The determination of crude proteins was made by mineralization process in the flasks, the distillation by machine VELP® *Scientifica* UDK 127 device, and titration relatively with the Kjeldahl method according to the AOAC (2002). Total lipids were determined by the method of Soxhlet through the solubilization of lipids in an organic solvent, hexane. The samples weighed in Whatman paper and placed in extraction inners of the balloons ramp heating Bistabil BRAND 6 positions and heated for 12 h and then weighed (AOAC, 2002). Total ash was determined by the weight difference by incinerate the samples in the oven electronic Heraeus (T 5042, Germany) at 550°C for 24 h carbohydrates by the difference between dry matter and (crude proteins+ total ash + fats+ total fibers). Energy was calculated by

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using carbohydrates, fats and crude proteins contents (Italian law, 1993). Minerals as calcium, magnesium, potassium, sodium, copper, iron, manganese, zinc, phosphorus are determined by flame atomic absorption spectrometry after mineralization of parallel samples in microwave mineralization (Milestone model START-D, Italy) with power of 800 W. The dry matter was determined by the method of drying according to the AOAC directions (AOAC, 2002). Samples of known weight were placed at 105°C for 48 h. The water content was calculated by the difference of dry matter obtained before and after drying.

Microbiological analysis

Sanitary quality of samples (*M. oleifera* powder, *P. ostreatus* raw powder, and mixture powders of *M. oleifera* and *P. ostreatus* cooked) were analyzed by identifying microorganisms including thermoresistants coliforms, *Escherichia coli*, *Salmonella* and Fungi commonly used as indicators of sanitary quality of water and foods (NF V08-060, 2009; NF ISO 16649-2, 2001; NF V08-061, 2009; NF ISO 21527-2, 2008). The detection of *Salmonella* was investigated according to the standard method of NF EN ISO 6579 (2002). A portion of each 25 g of mixture product was placed in 225 ml of buffered peptone water for pre-enrichment. The homogenized solution was incubated at 37°C for 24 h. Then, 0.1 ml of the solution was added in a tube containing 10 ml of Rappaport Vassiliadis broth (Biolife, Italy) incubated at 42°C for 24 h. The selective isolation was realized on medium Hektoen agar (Biolife, Italy). After researching thermoresistants coliforms, *Escherichia coli* were searched at 44°C for 24 h. For each tube of 10 ml of *EPSI* (*Eau Peptonée Sans Indole*), one colony was plated (NF ISO 16649-2, 2001). According to the standard method, Sulfite-reducer Anaerobe in mixture product was carried out by conventional methods NF V08-061(2009) based on the use of Tryptone Sulfite Néomycine medium (Biolife, Italy).

Data analysis

All experimental results are the averages of two parallel measurements (means \pm SD). Quantitative data were expressed as means and standard deviation (SD) of at least 2 measurements. Each experimental set was compared with one way analysis of variance (ANOVA) procedure using Statistical Package for Social Sciences (SPSS) version 20 (SPSS Inc., Chicago, IL, USA). Duncan's new multiple range test was used to determine the differences of means. P values <0.05 were regard as significant.

RESULTS

The macronutrients levels are presented in Table 1. The highest content of carbohydrates was 25.13 ± 0.20 mg for *P. ostreatus*, 36.81 ± 0.21 mg was the highest value of crude proteins in *M. oleifera* powder. Raw *P. ostreatus* had the highest content in fibers. The most abundant macronutrients in the mixture were crude proteins followed by fats, fibers and carbohydrates.

As can be seen in the Table 2, *M. oleifera* had the highest content of calcium, magnesium, manganese and sodium. Others minerals (iron, potassium, zinc, copper and phosphorus) were abundant in raw *P. ostreatus*. The most abundant mineral in the mixture was potassium followed by phosphorus, calcium and magnesium.

As can be concluded from data present in Table 3, almost elements contents except sodium could cover minimum 20% of DRI for children (1 to 8 years). For some nutrients, the levels were more than 100% of DRI.

Some minerals covered a minimum of 20% DRI while others contained more than 100% of children's DRI within the age group 9-18 years (Table 4). This is valid for adults who had more than 100% of crude proteins, iron and manganese (Table 5).

As can be concluded from data present in tables, almost elements contents could cover more than 20% of DRI of all groups. For some nutrients (proteins, iron, manganese), we have noticed more than 100% of DRI.

According to referenced standards, we didn't find any pathogens in the product (Table 6).

DISCUSSION

The dried leaves of *M. oleifera* and *P. ostreatus* are rich in macronutrients and minerals. According to Breene (1990), Çokuner and Özdemir (2000), Bénissan et al. (2012), proteins content in 100 g dried matter of *P. ostreatus* ranged from 19 to 39 g; for *M. oleifera* average amounts 35 g. In this study, the protein content is in conformity with Shin et al. (2007). Watanabe et al. (1994) found that the carbohydrates value of *P. ostreatus* as 47.9 g in 100 g dry matter which is higher than this study, however, Alam et al. (2008) found that a value as 37.8 g which is also not compatible with result 16.70 g in 100 g dry matter. This difference might be due to the difference of environment factors. The results are similar those of Moyo et al. (2011) who worked on nutritional characterization of *M. oleifera* leaves. The most abundant mineral in both species is potassium 1566.8 mg followed by phosphorus 318.5 mg and calcium 284.3 mg and the least content was copper with 0.53 mg.

According to Daily Reference Intakes in the Essential Guide of Nutrients Requirements (EGNR) (2006), sodium contents is low and the great amount of potassium suggest the utilization of this mixture in anti-hypertensive diet because of the fact, potassium from fruits and vegetables can lower blood pressure. These results and observations are similar those of Manzi et al. (1999). Calcium was observed to be higher compared with other plant sources (Nkafamiya et al., 2010). It plays a key role in bone health. In fact, more than 99% of total body calcium found in the teeth and bones. Calcium deficiency can result from inadequate intake or poor intestinal absorption and can cause osteopenia, osteoporosis, and an increased risk of fractures (EGNR, 2006). Interestingly too, iron, commonly deficient in many plant-based diets, which is a necessary component of hemoglobin and myoglobin for oxygen transport and cellular processes of growth and division (EGNR, 2006), an essential trace element for normal functioning of the central nervous

Table 1. Macronutrients contents of the dietary supplement FMP16 (g/100 g DM).

Sample	Total ash	Fats	Crude proteins	Fibers	Carbohydrates	Total Sugar	^a Energy
MO	8.77± 0.02	28.5±0.7	36.81±0.2	09.2±0.2	16.7±0.6	13±0.4	470±4
PO	5.71±0.04	27.37±0.54	28.97±0.06	12.83±0.33	25.13±0.20	12.86±0.61	463±4
MO+PO	6.98±0.33	22.71±0.05	35.08±0.160	20.96±1.425	14.28±1.310	11.78±0.145	402±4

^akcal/100 g DM. Values are means± standard deviation for two repetitions. FMP16: Code of product; DM, Dry matter; MO, Moringa oleifera leaves powder; PO, Pleurotus ostreatus raw powders; MO+PO, M. oleifera leaves powder + Pleurotus ostreatus cooked powder.

Table 2. Content of minerals in the prepared dietary supplement FMP16 (mg/100 g DM).

Sample	Iron	Calcium	Magnesium	Manganese	Copper	Potassium	Zinc	Sodium	Phosphorus
MO	20.96±0.01	583.3±8.05	306.19±0.40	5.18±0.00	0.48±0.01	1675.52±7.07	1.31±0.01	96.6	9.76±0.02
PO	24.3±0.07	21.22±0.63	86.35±0.09	0.74±0.00	0.66±0.00	2240.43±3.49	5.35±0.01	68.1	1693.57±3.41
PO+MO	19.66±0.07	284.33±1.40	253.14±0.45	3.70±0.02	0.53±0.01	1566.83±10.23	4.36±0.02	79.3	318.55±1.72

FMP16, Code of product; DM, Dry matter; MO, M. oleifera leaves powder; PO, P. ostreatus raw powders; MO+PO, M. oleifera leaves powder + P. ostreatus cooked powder. Values are means± standard deviation for two repetitions.

Table 3. Fulfillment of recommendation (Daily Reference Intakes) by the dietary supplement FMP16 (mg/100 g DM) for children (1-8 years)*.

Elements	DRI*	Contents of FMP16 (/100 g)	Percentages of DRI covered by FMP16 (%)
Crude proteins	20	35.08	175
Carbohydrates	65	14.28	22
Fats	ND	22.71	ND
Fibers	19	20.96	110
Magnesium	80	253.14	316
Phosphorus	460	318.55	69
Copper	1.5	0.53	35
Iron	7	19.66	280
Manganese	1.2	3.70	300
Zinc	3	4.36	145
Potassium	3800	1566.83	41
Calcium	700	284.33	40
Sodium	1200	79.3	6

DRI, Daily reference intakes; crude proteins, carbohydrates, fats, fibers (g/day); magnesium, phosphorus, iron, manganese, zinc, potassium, copper, calcium, sodium (mg/day); ND, Not determined.* Source: *EGNR*, Essential Guide to Nutrients Requirements (2006).

Table 4. Fulfillment of recommendation (Daily Reference Intakes) by the dietary supplement FMP16 (mg/100 g DM) for children (9 to 18 years)*.

Elements	DRI*	Contents of FMP16 (/100 g DM)	Percentages of DRI covered by FMP16 (%)
Crude proteins	30	35.08	117
Carbohydrates	65	14.28	22
Fats	ND	22.71	ND
Fibers	38	20.96	55
Magnesium	410	253.14	60
Phosphorus	1250	318.55	25
Copper	0.7	0.53	75
Iron	15	19.66	130
Manganese	1.9	3.70	190
Zinc	5	4.36	87
Potassium	4500	1566.83	34
Calcium	800	284.33	35
Sodium	1200	79.3	6

DRI, Daily reference intakes; crude proteins, carbohydrates, fats, fibers (g/day); magnesium, phosphorus, iron, manganese, zinc, potassium, copper, calcium, sodium (mg/day) – ND, Not determined; * Source: EGNR, Essential Guide to Nutrients Requirements (2006).

Table 5. Fulfillment of recommendation (Daily Reference Intakes) by the dietary supplement FMP16 (mg/100 g DM) for adults.

Elements	DRI*	Contents of FMP16 (/100 g DM)	Percentages of DRI covered by FMP16 (%)
Crude proteins	35	35.08	100
Carbohydrates	65	14.28	22
Fats	ND	22.71	ND
Fibers	38	20.96	55
Magnesium	420	253.14	60
Phosphorus	700	318.55	45
Copper	0.9	0.53	58
Iron	18	19.66	110
Manganese	2.3	3.70	160
Zinc	11	4.36	40
Potassium	4700	1566.83	33
Calcium	1200	284.33	23
Sodium	1500	79.3	5

DRI, Daily reference intakes; crude proteins, carbohydrates, fats, fibers (g/day); magnesium, phosphorus, iron, manganese, zinc, potassium, copper, calcium, sodium (mg/day) – ND, Not determined.*Source: EGNR, Essential Guide to Nutrients Requirements (2006).

system and in the oxidation of carbohydrates, proteins and fats (Umar et al., 2007), was found in abundance in these two species. These results are comparable to data published by Moyo et al. (2011). Results from this study had higher levels of zinc 4.36 mg/100 g. These results corroborate studies by Barminas et al. (1998), who reported 25.5 mg/kg in dried *Moringa* leaves. Zinc has been shown to boost the immune system, is also required for cell reproduction and growth especially sperm cells (Brisibe et al., 2009). Raw *P. ostreatus* and *M. oleifera*

leaves were analyzed separately, then the mixture was done by mixing both, but in this case, *P. ostreatus* were cooked. According to Manzi et al. (2004), the effect of the cooking process is explained as a decrease of the nutrients contents of raw sample in the water, and consequently their concentration due to thermal degradation. The cooking process might affect the nutritional value by decreasing minimum 70% of his content. However, this decrease does not affect fibers which stay constant. Also, according to Barminas et al.

Table 6. Microbiological quality of the dietary supplement FMP16.

Strains	Results	References values (Unit)
Thermo-resistant coliforms	Absence	10/g-100/g ^a
<i>E. coli</i>	Absence	10/g-100/g ^b
Sulfito- reducer Anaerobe	Absence	<10/g ^c
<i>Salmonella</i>	Absence	Absence/25g ^d
Fungi	Absence	10/g-100/g ^e

a, NF V08-060 (2009); b, NF ISO 16649-2 (2001); c, NF V08-061(2009); d, NF EN ISO 6579 (2002); e, NF ISO 21527-2 (2008).

(1998) and Broin (2006), the mode of conservation and time between collection and analysis might influence the nutritional composition; it is worth mentioning that these analyses were completed 6 months after collection and transformation.

According to the daily recommendations in the EGNR (2006), by the Institute of Medicine of the National Academies, vitamins and minerals requirements (FAO, 2004), 100 g of this dietary supplement composed by *M. oleifera* and *P. ostreatus* might cover many recommended daily reference intakes of minerals and macronutrients. Percentages covered by each element are represented in Tables 3 to 5; showed that the dietary supplement is a good source of minerals and macronutrients more than many others plants which are used like food supplement (Bénissan et al., 2012; Moyo et al., 2011; Maiga et al., 2005).

Conclusion

In conclusion, mushrooms such as *P. ostreatus* could be excellent food that can be used in alimentation for malnutrition problem in sub-Saharan Africa for their high contents in macronutrients and minerals. In this study confirmed the high nutritional quality of *M. oleifera* leaves which can be used to improve health and reduce malnutrition in the world. The data derived from nutritional value of this mixture of *M. oleifera* and *P. ostreatus* are clear indications that these species are rich in nutrients and had potential to be used as a food supplement and a promising dietary supplement that may overcome protein-energy malnutrition problem in the third world. For that reason, our dietary supplement composed of these two species could be a very good source of nutrients for reduce malnutrition rate.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Effect of storage on lutein levels of smooth cayenne (*Ananas comosus*) grown in different counties of Kenya

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Epidemiological studies have repeatedly shown the apparent association between high fruit intake and lower incidence of chronic degenerative diseases. The chemo protective properties of fruits have been partly attributed to the antioxidants including phenolic compounds and carotenoids. With respect to the latter, no information is available for the smooth cayenne; a common variety of *Ananas comosus* grown in various counties of Kenya. Of particular concern is how duration under room temperature storage, will affect the carotenoids based on the challenging aspect of the perishability of the fruit. The levels of Lutein were investigated during storage of fruit samples of smooth cayenne sourced from Kiambu, Homabay, Kilifi, Kericho and Nyamira Counties, Kenya and analyzed within 7 days of storage under room temperature (21 to 25°C). High performance liquid chromatography method and one way ANOVA were employed instrumental and statistical analysis, respectively. The lutein levels ranged between 107.52 ± 1.25 and 233.55 ± 5.77 $\mu\text{g}/100\text{g}$ being categorized as sufficient. Findings not only showed that the levels differed significantly among samples but as well increased during storage ($p < 0.05$), hence this show the effect of storage on the nutritional value of the fruit. While the fruit consumption should be promoted for the sufficient levels of lutein which can be useful in addressing chronic degenerative diseases, the longer the storage of the fruit will enhance the levels of the carotenoid.

Key words: *Ananas comosus*, smooth cayenne, lutein, carotenoids, chronic degenerative diseases.

INTRODUCTION

A significant proportion of global deaths are attributed to chronic degenerative diseases (CDDs) which include cancer and cardiovascular disease (Francesco and Monica, 2016). In 2012, CDDs caused approximately 38 million (68%) of the worlds' 56 million deaths (WHO,

2014). Fundamentally, the diseases are related to life styles which include poor diet thus compromising quality of life and as well may cause premature death and disability (WHO/FAO, 2003). While diet and nutrition is coming to the fore as the major determinant of CDDs,

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epidemiological studies shows apparent association between high fruit consumption and their low incidence (Francesco and Monica, 2016; Heiner et al., 2012). The underlying reason is that fruits and vegetables contain significant amounts of biologically active phytochemical compounds such as carotenoids (β -carotene, lycopene and lutein) compounds, whose levels generally correlate with their antioxidant activity (Corral-Aguayo et al., 2008; Maria et al., 2011). In other words, fruits including *A. comosus* L (Pineapple) should be promoted for consumption to address CDDs.

Pineapple (*A. comosus* L) is a tropical fruit which is a native of southern Paraguay and Brazil (Walter et al., 2014; Annemarie, 2012). Kenya appears in the list of the main pineapple producers in the world and is the leading producer in East Africa (Annemarie, 2012; USAID, 2013). Pineapple fruit is of high economic importance and the commercial variety of pineapple commonly grown in Kenya is the smooth cayenne. Del monte's farm in Kiambu County is the leading producer in Kenya but small-scale growing is on the increase in Homabay, Kilifi, Nyamira and Kericho counties (Walter et al., 2014).

Depending on the variety, commercially ripe pineapple weighs between 0.9 to 4 kg. Ripe pineapples can be eaten fresh, or processed into jam, juice, canned or dried (Annemarie, 2012). Like any other, the carotenoid levels of this fruit are associated with; the type of cultivar, agronomic conditions, growing season, pH, moisture content, stage of ripeness, postharvest, and storage conditions (Wang and Lin, 2000; Kalyani, 2009).

Studies have concentrated on the tropical fruits in particular on their levels of antioxidants and antioxidant activity. The lack of information for the smooth cayenne *A. comosus* grown in Kenya presented a gap for study. In addition, carotenoids chemistry is generally challenging as they are sensitive to others like air, heat, acid, oxygen and light. Pineapple fruits when ripe can be stored in bulk for consumption over days; this is a common traditional practice. As to whether storage would have effect on the lutein level this information that is generally not scientifically investigated. Thus, there is an impetus to investigate the effect of storage on the levels of lutein in the smooth cayenne *A. comosus* grown in Counties of Kenya.

MATERIALS AND METHODS

Chemicals and reagents

Calcium carbonate was from Merck chemical supplies (Damstadt, Germany). Lycopene, Lutein and β -carotene standards were obtained from Sigma-Aldrich Chemical Corporation, USA. Acetonitrile, methanol, deionised water and dichloro methane (all HPLC grade) were obtained from Sigma-Aldrich Chemical Corporation. All the other chemicals used including the solvents, were of analytical grade purchased from Sigma-Aldrich Chemical

Corporation.

Sampling and sample preparation

Purposive sampling technique was employed to harvest commercially mature fruits which were 120 to 125 days from flowering, quarter-yellow in colour and weighing between 3 to 3.5 kg, from farms in Olare (Homabay), Ngoina (Kericho), Kanyoni (Kiambu), Ekonge (Nyamira) and Chamari (Kilifi). The pineapples sampled were ensured to have no external defect or damage (indicated by the secretion of a fluid).

The samples obtained from the five regions were coded appropriately and packed into labeled sisal bags, then transported to Kenyatta university chemistry laboratory within 24 h of collection where the samples were stored on clean shelves under room temperature (21 to 25°C), awaiting determination of changes in the levels of lutein over 7 days of storage. The fruit samples were identified and authenticated by the herbarium curator at National Museums of Kenya, Nairobi. An accession number (NMB/BOT/CTX/1/2) was deposited in the herbarium section of the museum.

Extraction, identification and quantification of carotenoids

One hundred grams (100 g) of sample was homogenized in an electric blender for 1 min, in the dark-room of the research laboratory. 20 g of homogenized paste was measured and 100 mL of acetone:methanol:diethylether mixture (5:3:2) containing 0.5% butylated hydroxytoluene was used to extract carotenoids for 2 min in separating funnel. The extraction was repeated three times to ensure that maximum extraction occur. Calcium carbonate powder was added to neutralize plant acids and avoid *cis/trans* isomerisation of carotenoids during extraction. The mixture was filtered using Buchner funnel and small portions of the extract were added into 50 ml of diethyl ether and n-hexane mixture (3:1) in a separating funnel for partitioning, then carefully washed six times using distilled water to remove all acetone and methanol.

Anhydrous sodium sulphate was added to the sample portion in other to dry it. Then the samples was concentrated to dryness in rotary evaporator (BUCHI, Rotavapor R-210/R-215, Germany) to near dryness at temperature 30°C under nitrogen environment. The samples were re-dissolved in 6 mL methanol and packed in HPLC vials in nitrogen atmosphere. Identification and quantification was done by HPLC procedure (Matsumoto et al., 2007).

One microgram (1 μ g) aliquot of sample was introduced into the HPLC instrument (Prominence LC-20A). The instrument consists of oven (CTO-10ASVP), a LC-20AD pump and a C_{18} column (250 x 4.6 mm id, 5 μ m particle size). Mobile phase are made up of acetonitrile, methanol and dichloromethane (70:10:20) with a flow rate of 20 min. The column temperature was maintained at 30°C. The carotenoids were identified on the basis of retention time, by comparing spectra to standards analyzed under the same conditions. The carotenoids were quantified using single point calibration and the results are expressed in μ g/100 g of sample.

Data analysis

Lutein levels were determined daily for 7 days, where experimental measurements were carried out in triplicates and expressed as means of three replicate analyses \pm standard deviation, compared by One-way ANOVA test at 95% confidence level using SPSS(Chicago, IL) statistical software package (SPSS for Windows, version XII, 2004). Differences at $P < 0.05$ were considered as

Table 1. Mean concentrations of lutein in pineapples from Homabay County Kenya, stored at room temperature for 7 days.

Days	Concentrations ($\mu\text{g}/100\text{ g}$) $\bar{X} \pm SE ; n=3$	% increase change by day	% change wrt to day 1
1	107.52 \pm 1.25 ^{Aa}	-	-
2	133.45 \pm 1.73 ^{Ab}	24.12	24.12
3	161.91 \pm 4.04 ^{Cc}	21.33	50.59
4	166.34 \pm 1.39 ^{Dc}	2.74	54.71
5	173.91 \pm 1.15 ^{Cd}	4.55	61.75
6	174.72 \pm 1.73 ^{Cd}	0.47	62.50
7	228.21 \pm 6.35 ^{Be}	30.61	112.25

p-value<0.001, Mean values with different small letter(s) within the same column are significantly different (SNK, $\alpha=0.05$).

Table 2. Mean concentrations of Lutein in pineapples from Kilifi County Kenya, stored at room temperature for 7 days.

Days	Concentrations ($\mu\text{g}/100\text{ g}$) $\bar{X} \pm SE ; n=3$	% increase change by day	% change wrt to day 1
1	138.79 \pm 0.81 ^{Da}	-	-
2	151.07 \pm 1.73 ^{Cb}	8.85	8.85
3	152.20 \pm 1.15 ^{Bb}	0.75	9.66
4	165.34 \pm 1.15 ^{Dc}	8.63	19.13
5	179.44 \pm 1.27 ^{Dd}	8.53	29.29
6	231.84 \pm 5.77 ^{Ee}	29.20	67.04
7	233.55 \pm 5.77 ^{Be}	0.74	68.28

p-value<0.001, Mean values with different small letter(s) within the same column are significantly different (SNK, $\alpha=0.05$).

Table 3. Mean concentrations of Lutein in pineapples from Kericho County Kenya stored at room temperature for 7 days.

Days	Concentrations ($\mu\text{g}/100\text{ g}$) $\bar{X} \pm SE ; n=3$	% increase change by day	% change wrt to day 1
1	144.52 \pm 1.15 ^{Ea}	-	-
2	145.32 \pm 1.73 ^{Ba}	0.55	0.55
3	153.29 \pm 1.73 ^{Bb}	5.48	6.07
4	153.84 \pm 1.15 ^{Ab}	0.36	6.45
5	166.66 \pm 1.15 ^{Bc}	8.33	15.32
6	165.25 \pm 1.73 ^{Bc}	-	14.34
7	182.90 \pm 1.73 ^{Ad}	10.68	26.56

p-value<0.001, Mean values with different small letter(s) within the same column are significantly different (SNK, $\alpha=0.05$).

significantly different and SNK-test at $\alpha=0.05$ indicate differences among means.

RESULTS AND DISCUSSION

Lutein content in pineapples

Tables 1 to 5 present the levels of lutein in pineapples obtained from Homabay, Kilifi, Kericho, Nyamira and

Kiambu Counties of Kenya, respectively. The trends of lutein concentration in pineapples, from the Counties with storage at room temperature for 7 days are shown in Figure 1.

Substantial levels of lutein were found in pineapples obtained from all Counties, ranged between 107.52 \pm 1.25 $\mu\text{g}/100\text{ g}$ and 233.55 \pm 5.77 $\mu\text{g}/100\text{ g}$. There was a significant difference ($p<0.001$) in the level of lutein in 5 samples of pineapples. On day one of analysis, Kericho

Table 4. Mean concentrations of Lutein in pineapples from Nyamira County Kenya stored at room temperature for 7 days.

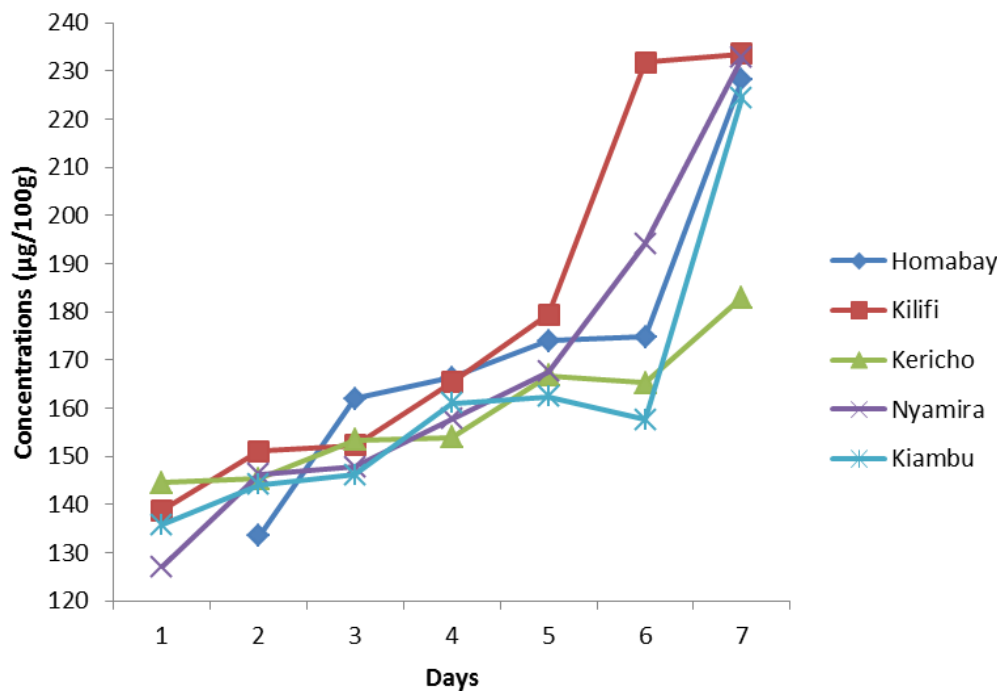
Days	Concentrations ($\mu\text{g}/100\text{ g}$) $\bar{X} \pm SE ; n=3$	% increase change by day	% change wrt to day 1
1	127.00 \pm 1.15 ^{Ba}	-	-
2	146.25 \pm 1.15 ^{Bb}	15.16	15.16
3	147.73 \pm 1.15 ^{Ab}	1.01	16.32
4	157.67 \pm 1.15 ^{Bc}	6.73	24.15
5	167.58 \pm 0.58 ^{Bd}	6.29	31.95
6	194.15 \pm 2.89 ^{De}	15.86	52.87
7	232.75 \pm 2.31 ^{Bf}	19.88	83.27

p-value<0.001 Mean values with different small letter(s) within the same column are significantly different (SNK, $\alpha=0.05$).

Table 5. Mean concentrations of Lutein in pineapples from Kiambu County Kenya stored at room temperature for 7 days.

Days	Concentrations ($\mu\text{g}/100\text{g}$) $\bar{X} \pm SE ; n=3$	% increase change by day	% change wrt to day 1
1	135.71 \pm 1.15 ^{Ca}	-	-
2	144.08 \pm 1.15 ^{Bb}	6.17	6.17
3	146.17 \pm 1.15 ^{Ab}	1.45	7.71
4	161.05 \pm 1.15 ^{Cd}	10.18	18.67
5	162.32 \pm 1.15 ^{Ad}	0.79	19.61
6	157.62 \pm 1.15 ^{Ac}	-	16.14
7	224.45 \pm 1.73 ^{Be}	42.40	65.39

p-value<0.001, Mean values with different small letter(s) within the same column are significantly different (SNK, $\alpha=0.05$).

**Figure1.** Trend of lutein in pineapples from Counties in Kenya over 7 days storage period.

sample had the highest lutein content (144.52 ± 1.15 $\mu\text{g}/100$ g) and Homabay the lowest (107.52 ± 1.25 $\mu\text{g}/100$ g). On day 7 of analysis, Kilifi sample had the highest content (233.55 ± 5.77 $\mu\text{g}/100$ g) and Kericho sample the lowest (182.90 ± 1.73 $\mu\text{g}/100$ g). The differences in content of lutein in common cultivar may be attributed to the presence of difference genes in each of the fruits investigated (Murkovic et al., 2002). In general, Kenyan grown pineapples contain significant levels of lutein though lower than levels reported in broccoli (2358 $\mu\text{g}/100$ g wet wt), kale (6390 $\mu\text{g}/100$ g wet wt), carrot (280 $\mu\text{g}/100$ g wet wt) and spinach (3920 $\mu\text{g}/100$ g wet wt) (Huck et al., 2000).

It was also observed that the level of lutein increased significantly ($p < 0.001$) as the fruits were stored from day 1 to 7. Similar observations were noted by Kalyani (2009) in fresh pineapples which showed an increase in lutein content of (27.20 to 40.32 μg). Kalyani (2009) attributed this increase to factors such as pH and moisture. Increase in pH during storage of pineapple increases the lutein content in the fruit, while decrease in moisture content increases the lutein levels. The decrease in moisture content lowers the mobility of reactants and catalysts hence reducing the rate of oxidation (Lavelli et al., 2007). The percentage increase in the levels of lutein may also be attributed to the presence of significant levels of phytoene synthase enzyme, which is involved in the biosynthesis of the carotenoid in the pineapple fruits investigated (Bitton and Khachik, 2009; Abdul-Hammed et al., 2014). The overall gain in lutein levels during storage may also be attributed to the conversion of other carotenoids to lutein or to the presence of ϵ -cyclase and ϵ -hydroxylase enzymes in the fruits (Bitton and Khachik, 2009). This finding is in agreement with earlier researchers (Hena et al., 2016)

Conclusion

1. Lutein was present in significant amount, with significant difference in levels of lutein in the fruit sample sourced from the five Counties.
 2. There was significant increase in the level of lutein as the pineapple fruits were stored from day 1 to 7.
 3. Increase in lutein levels, showed that continuous ripening of the fruit during storage could be a way of increasing lutein content in the pineapple fruit after harvesting.
- These findings propose that, proper storage condition and period should be sought to obtain the maximum nutritional and medicinal benefits of pineapple fruits.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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